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09/509032
430 Rec'd PCT/PTO 21 MAR 2000

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Title: **NEW AMIDINO DERIVATIVES AND THEIR USE AS THROMBIN INHIBITORS**

Reference: H 2059-1 WO

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09/509032

430 Rec'd PCT/PTO 21 MAR 2000

NEW AMIDINO DERIVATIVES AND THEIR USE AS THROMBIN INHIBITORS

Field of the Invention

This invention relates to novel pharmaceutically useful compounds, in particular compounds that are, or are prodrugs of, competitive inhibitors of trypsin-like serine proteases, especially thrombin, their use as medicaments, pharmaceutical compositions containing them and synthetic routes to their production.

Background

Blood coagulation is the key process involved in both haemostasis (i.e. the prevention of blood loss from a damaged vessel) and thrombosis (i.e. the formation of a blood clot in a blood vessel, sometimes leading to vessel obstruction).

Coagulation is the result of a complex series of enzymatic reactions. One of the ultimate steps in this series of reactions is the conversion of the proenzyme prothrombin to the active enzyme thrombin.

Thrombin is known to play a central role in coagulation. It activates platelets, leading to platelet aggregation, converts fibrinogen into fibrin monomers, which polymerise spontaneously into fibrin polymers, and activates factor XIII, which in turn crosslinks the polymers to form insoluble fibrin. Furthermore, thrombin activates factor V and factor VIII leading to a "positive feedback" generation of thrombin from prothrombin.

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By inhibiting the aggregation of platelets and the formation and crosslinking of fibrin, effective inhibitors of thrombin would be expected to exhibit antithrombotic activity. In addition, antithrombotic activity would be expected to be enhanced by effective inhibition of the positive feedback mechanism.

Further, it is known that administration of prodrugs of thrombin inhibitors may give rise to improvements in:

(a) certain pharmacokinetic properties after administration of; and

(b) the prevalence of certain side effects associated with,

those inhibitors.

Prior Art

The early development of low molecular weight inhibitors of thrombin has been described by Claesson in Blood Coagul. Fibrinol. (1994) **5**, 411.

Blombäck *et al* (in J. Clin. Lab. Invest. **24**, suppl. 107, 59, (1969)) reported thrombin inhibitors based on the amino acid sequence situated around the cleavage site for the fibrinogen Aα chain. Of the amino acid sequences discussed, these authors suggested the tripeptide sequence Phe-Val-Arg (P9-P2-P1, hereinafter referred to as the P3-P2-P1 sequence) would be the most effective inhibitor.

Thrombin inhibitors based on dipeptidyl derivatives with an α,ω-aminoalkyl guanidine in the P1-position are known from US Patent N° 4,346,078 and International Patent Application WO 93/11152. Similar, structurally related, dipeptidyl derivatives have also been reported. For example International Patent Application WO 94/29336 discloses

compounds with, for example, aminomethyl benzamidines, cyclic aminoalkyl amidines and cyclic aminoalkyl guanidines in the P1-position (International Patent Application WO 97/23499 discloses prodrugs of certain of these compounds); European Patent Application 0 648 780, discloses compounds with, for example, cyclic aminoalkyl guanidines in the P1-position.

Thrombin inhibitors based on peptidyl derivatives, also having cyclic aminoalkyl guanidines (e.g. either 3- or 4-aminomethyl-1-amidinopiperidine) in the P1-position are known from European Patent Applications 0 468 231, 0 559 046 and 0 641 779.

Thrombin inhibitors based on tripeptidyl derivatives with arginine aldehyde in the P1-position were first disclosed in European Patent Application 0 185 390.

More recently, arginine aldehyde-based peptidyl derivatives, modified in the P3-position, have been reported. For example, International Patent Application WO 93/18060 discloses hydroxy acids, European Patent Application 0 526 877 des-amino acids, and European Patent Application 0 542 525 O-methyl mandelic acids in the P3-position.

Inhibitors of serine proteases (e.g. thrombin) based on electrophilic ketones in the P1-position are also known. For example, European Patent Application 0 195 212 discloses peptidyl α-keto esters and amides, European Patent Application 0 362 002 fluoroalkylamide ketones, European Patent Application 0 364 344 α,β,δ-triketocompounds, and European Patent Application 0 530 167 α-alkoxy ketone derivatives of arginine in the P1-position.

Other, structurally different, inhibitors of trypsin-like serine proteases based on C-terminal boronic acid derivatives of arginine and isothiouronium analogues thereof are known from European Patent Application 0 293 881.

More recently, thrombin inhibitors based on peptidyl derivatives have been disclosed in European Patent Application 0 669 317 and International Patent Applications WO 95/35309, WO 95/23609, WO 96/03374, WO 96/25426, WO 96/31504, WO 97/02284, WO 97/46577, WO 96/32110, WO 98/06740, WO 97/49404 and WO 98/57932.

However, there remains a need for effective inhibitors of trypsin-like serine proteases, such as thrombin. There is a particular need for compounds which are both orally bioavailable and selective in inhibiting thrombin over other serine proteases. Compounds which exhibit competitive inhibitory activity towards thrombin would be expected to be especially useful as anticoagulants and therefore in the therapeutic treatment of thrombosis and related disorders.

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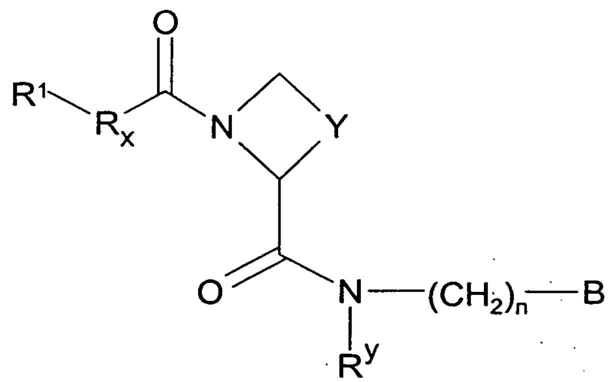
Disclosure of the Invention

According to the invention there is provided a compound of formula I,



I

wherein

R^1 represents H, C_{1-4} alkyl (optionally substituted by one or more substituents selected from cyano, halo, OH, $C(O)OR^{1a}$ or $C(O)N(R^{1b})R^{1c}$) or OR^{1d};

R^{1d} represents H, $C(O)R^{11}$, $SiR^{12}R^{13}R^{14}$ or C_{1-6} alkyl, which latter group is optionally substituted or terminated by one or more substituent selected from OR^{15} or $(CH_2)_qR^{16}$;

R^{12}, R^{13} and R^{14} independently represent H, phenyl or C_{1-6} alkyl;

R^{16} represents C_{1-4} alkyl, phenyl, OH, $C(O)OR^{17}$ or $C(O)N(H)R^{18}$;

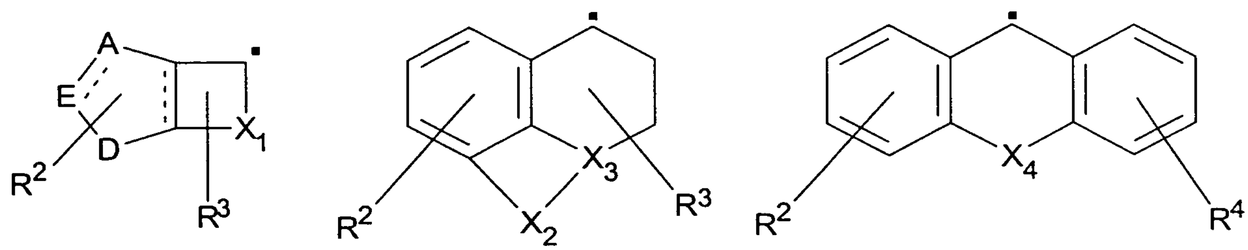
R^{18} represents H, C_{1-4} alkyl or $CH_2C(O)OR^{19}$;

R^{15} and R^{17} independently represent H, C_{1-6} alkyl or C_{1-3} alkylphenyl;

R^{1a}, R^{1b}, R^{1c}, R^{11} and R^{19} independently represent H or C_{1-4} alkyl; and

q represents 0, 1 or 2;

R_x represents a structural fragment of formula IIa, IIb or IIc,



IIa IIb IIc

wherein

the dotted lines independently represent optional bonds;

A and E independently represent O or S, CH or CH_2 (as appropriate), or N or $N(R^{21})$ (as appropriate);

D represents $-CH_2-$, O, S, $N(R^{22})$, $-(CH_2)_2-$, $-CH=CH-$, $-CH_2N(R^{22})-$, $-N(R^{22})CH_2-$, $-CH=N-$, $-N=CH-$, $-CH_2O-$, $-OCH_2-$, $-CH_2S-$ or $-SCH_2-$;

X_1 represents C_{2-4} alkylene; C_{2-3} alkylene interrupted by Z; $-C(O)-Z-A^1-$; $-Z-C(O)-A^1-$; $-CH_2-C(O)-A^1-$; $-Z-C(O)-Z-A^2-$; $-CH_2-Z-C(O)-A^2-$; $-Z-CH_2-C(O)-A^2-$; $-Z-CH_2-S(O)_m-A^2-$; $-C(O)-A^3$; $-Z-A^3-$; or $-A^3-Z-$;

X_2 represents C_{2-3} alkylene, $-C(O)-A^4-$ or $-A^4-C(O)-$;

X_3 represents CH or N;

X_4 represents a single bond, O, S, C(O), $N(R^{23})$, $-CH(R^{23})-$, $-CH(R^{23})-CH(R^{24})-$ or $-C(R^{23})=C(R^{24})-$;

A^1 represents a single bond or C_{1-2} alkylene;

A^2 represents a single bond or $-CH_2-$;

A^3 represents C_{1-3} alkylene;

A^4 represents C(O) or C_{1-2} alkylene;

Z represents, at each occurrence, O, $S(O)_m$ or $N(R^{25})$;

R^2 and R^4 independently represent one or more optional substituents

selected from C_{1-4} alkyl, C_{1-4} alkoxy (which latter two groups are optionally substituted by one or more halo substituent), methylenedioxy, halo, hydroxy, cyano, nitro, $S(O)_2NH_2$, $C(O)OR^{26}$, SR^{26}, $S(O)R^{26a}$, $S(O)_2R^{26a}$ or $N(R^{27})R^{28}$;

R^3 represents one or more optional substituents selected from OH, C_{1-4} alkoxy, C_{1-6} alkyl (optionally substituted by one or more halo group), or $N(R^{29a})R^{29b}$;

R^{25}, R^{29a} and R^{29b} independently represent H, C_{1-4} alkyl or $C(O)R^{30}$;

R^{26} represents H or C_{1-4} alkyl;

R^{26a} represents C_{1-4} alkyl;

R^{27} and R^{28} independently represent H, C_{1-4} alkyl or $C(O)R^{30}$, or together represent C_{3-6} alkylene, thus forming a 4- to 7-membered ring, which ring is optionally substituted, on a carbon atom that is α to the nitrogen atom, with an =O group;

R^{21}, R^{22}, R^{23}, R^{24} and R^{30} independently represent, at each occurrence, H or C_{1-4} alkyl;

Y represents CH_2, $(CH_2)_2$, CH=CH (which latter group is optionally substituted by C_{1-4} alkyl), $(CH_2)_3$, $CH_2CH=CH$ or $CH=CHCH_2$ (which latter three groups are optionally substituted by C_{1-4} alkyl, methylene, =O or hydroxy);

R^y represents H or C_{1-4} alkyl;

n represents 0, 1, 2, 3 or 4; and

B represents a structural fragment of formula IIIa, IIIb or IIIc

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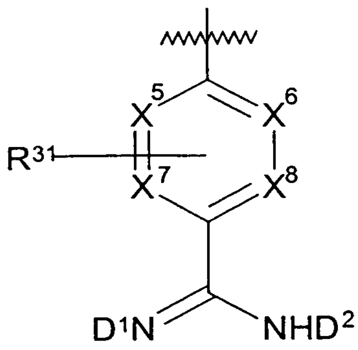
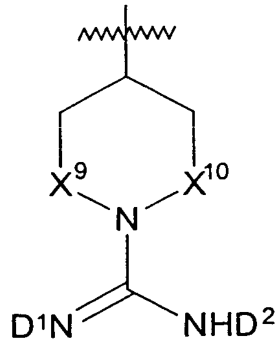
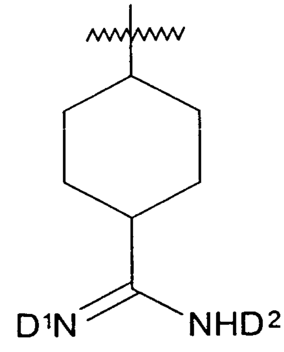


IIIa



IIIb



IIIc

wherein

X^5, X^6, X^7 and X^8 independently represent CH, N or N-O;

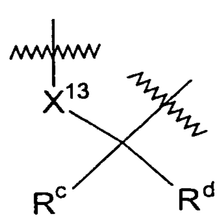
X^9 and X^{10} independently represent a single bond or CH_2;

R^{31} represents an optional substituent selected from halo, C_{1-4} alkyl (which group is optionally substituted by one or more halo group), $N(R^{32})R^{33}$, OR^{34} or SR^{35};

R^{32} and R^{33} independently represent H, C_{1-4} alkyl or $C(O)R^{36}$;

R^{34}, R^{35} and R^{36} independently represent H or C_{1-4} alkyl; and

one of D^1 and D^2 represents H, and the other represents H, OR^a, NHR^a, $C(=X^{11})X^{12}R^b$, or D^1 and D^2 together represent a structural fragment of formula IVa:-



IVa

R^a represents H or $-A^5[X^{14}]_n[C(O)]_rR^e$;

R^b represents $-A^5[X^{14}]_n[C(O)]_rR^e$;

A^5 represents, at each occurrence, a single bond or C_{1-12} alkylene (which alkylene group is optionally interrupted by one or more O, $S(O)_m$ and/or

$N(R^f)$ group, and is optionally substituted by one or more of halo, OH, $N(H)C(O)R^g$, $C(O)N(R^g)R^h$, C_{3-7}-cycloalkyl (which cycloalkyl group is optionally interrupted by one or more O, $S(O)_m$ and/or $N(R^f)$ group and/or is optionally substituted by one or more substituents selected from C_{1-6} alkyl, C_{1-6} alkoxy, halo, =O or =S), Het and C_{6-10} aryl (which aryl and Het groups are themselves optionally substituted by one or more substituents selected from C_{1-6} alkyl (optionally substituted by one or more halo substituent), C_{1-6} alkoxy, halo, cyano, $C(O)OR^g$, $C(O)N(R^g)R^h$ and $N(R^f)R^g$));

R^c and R^d both represent H; or one of R^c and R^d represents H or C_{1-7} alkoxy and the other represents C_{1-7} alkyl (which alkyl group is optionally interrupted by one or more O atoms); or R^c and R^d together represent C_{3-8} cycloalkyl, which cycloalkyl group is interrupted by one or more O, $S(O)_m$ and/or $N(R^f)$ group;

R^e represents, at each occurrence, H, C_{1-12} alkyl (which alkyl group is optionally interrupted by one or more O, $S(O)_m$ and/or $N(R^f)$ group, and/or is optionally substituted by one or more substituents selected from halo, OH, $N(H)C(O)R^g$ and $C(O)N(R^g)R^h$), A^7-C_{3-7}-cycloalkyl (which cycloalkyl group is optionally interrupted by one or more O, $S(O)_m$ and/or $N(R^f)$ group and/or is substituted by one or more substituents selected from C_{1-6} alkyl, C_{1-6} alkoxy, halo, =O and =S), A^7-C_{6-10} aryl or A^7-Het (which aryl and Het groups are optionally substituted by one or more substituents selected from C_{1-6} alkyl (optionally substituted by one or more halo substituent), C_{1-6} alkoxy, halo, cyano, $C(O)OR^g$, $C(O)N(R^g)R^h$ and $N(R^f)R^g$);

A^7 represents a single bond or C_{1-7} alkylene (which alkylene group is optionally interrupted by one or more O, $S(O)_m$ and/or $N(R^f)$ group, and/or are optionally substituted by one or more of halo, OH, $N(H)COR^g$ and $CON(R^g)R^h$);

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Het represents, at each occurrence, a five- to ten-membered heteroaryl group, which may be aromatic in character, containing one or more nitrogen, oxygen or sulphur atoms in the ring system;

n and r independently represent 0 or 1;

X^{11}, X^{12} and X^{14} independently represent O or S;

X^{13} represents O or $N(R^f)$;

R^f represents, at each occurrence, H, C_{1-4} alkyl or $C(O)R^g$;

R^g and R^h independently represent, at each occurrence, H or C_{1-4} alkyl; and

m represents, at each occurrence, 0, 1 or 2;

or a pharmaceutically acceptable salt thereof;

provided that:

(a) A and E do not both represent O or S;

(b) E and D do not both represent O or S;

(c) when R^1 represents OR^{1d} and X_1 represents $-C(O)-Z-A^1$, $-Z-CH_2-S(O)_m-A^2-$ or $-Z-C(O)-Z-A^2$, then A^1 or A^2 (as appropriate) do not represent a single bond;

(d) when X_4 represents $-CH(R^{23})-$, R^1 does not represent OH;

(e) when A^5 represents a single bond, then n and r both represent 0;

(f) when A^5 represents C_{1-12} alkylene, then n represents 1;

(g) when A^5 represents $-CH_2-$, n is 1 and r is 0, then R^e does not represent H; and

(h) the compound is not:-

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Pro-Pab;

(*R*)- or (*S*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Pro-Pab;

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab x HOAc;

(*R*)- or (*S*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab;

1-hydroxy-5-methoxytetralin-1-yl-C(O)-Aze-Pab x HOAc;

1-hydroxy-5,7-dimethyltetralin-1-yl-C(O)-Aze-Pab x HOAc;

1-hydroxy-7-aminotetralin-1-yl-C(O)-Aze-Pab x HOAc;

1-hydroxytetralin-1-yl-C(O)-Aze-Pab x HOAc;

7-methoxytetralin-1-yl-C(O)-Aze-Pab x HOAc;

(*R*)- or (*S*)-7-methoxy-1-methyltetralin-1-yl-C(O)-Aze-Pab;

4-hydroxy-6-methoxychroman-4-yl-C(O)-Aze-Pab x OAc;

(*S*)- or (*R*)-1-hydroxy-4-methoxyindan-1-yl-C(O)-Aze-Pab;

1-hydroxy-5-methoxytetralin-1-yl-C(O)-Aze-Pab(OH);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(OH);

4-hydroxy-6-methoxychroman-4-yl-C(O)-Aze-Pab(OH);

4-hydroxy-6-methoxychroman-4-yl-C(O)-Aze-Pab(OMe);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab-(C(O)OCH$_2$CCl$_3$);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab-(C(O)OCH$_2$CH$_3$);

7-methoxy-1-allyltetralin-1-yl-C(O)-Aze-Pab x HOAc;

(*S*)- or (*R*)-1-hydroxy-7-chlorotetralin-1-yl-C(O)-Pro-Pab;

1-*n*-propyl-7-methoxytetralin-1-yl-C(O)-Aze-Pab x HOAc;

6-chloro-4-hydroxychroman-4-yl-C(O)-Aze-Pab x HOAc;

4-hydroxychroman-4-yl-C(O)-Aze-Pab x HOAc;

6,8-dichloro-4-hydroxychroman-4-yl-C(O)-Aze-Pab x HOAc;

6-fluoro-4-hydroxychroman-4-yl-C(O)-Aze-Pab x HOAc;

4-hydroxy-6-methylchroman-4-yl-C(O)-Aze-Pab x HOAc;

8-chloro-4-hydroxy-6-methoxychroman-4-yl-C(O)-Aze-Pab x HOAc;

6-chloro-4-hydroxy-8-methylchroman-4-yl-C(O)-Aze-Pab x HOAc;

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-C(O)-*i*-Pr);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-C(O)-Et);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-C(O)-Ch);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-allyl);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-Bzl);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab-(CO-O-methallyl);

1-hydroxy-7-aminotetralin-1-yl-C(O)-Aze-Pab(OH);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-Val);

(*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-(Me)Pab; or

9-hydroxyfluoren-9-yl-C(O)-Aze-Pab x HOAc,

which compounds are referred to hereinafter as "the compounds of the invention".

The compounds of the invention may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention. Particular tautomeric forms of compounds of the invention that may be mentioned include those connected with the position of the double bond in the amidine functionality in the structural fragment B, and the position of D^1 and D^2, when one of these does not represent H. Further, it will be appreciated by those skilled in the art that, in the structural fragment of formula IIa, the optional double bonds, may, in conjunction with certain identities of substituent D, render the ring bearing A, E and D aromatic in character.

The compounds of formula I may also contain one or more asymmetric carbon atoms and may therefore exhibit optical and/or diastereoisomerism. All diastereoisomers may be separated using conventional techniques, e.g. chromatography or fractional crystallisation. The various stereoisomers may be isolated by separation of a racemic or other mixture of the

compounds using conventional, e.g. fractional crystallisation or HPLC, techniques. Alternatively the desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, for example with a homochiral acid followed by separation of the diastereomeric derivatives by conventional means (e.g. HPLC, chromatography over silica). All stereoisomers are included within the scope of the invention.

The term "aryl" includes phenyl, naphthyl and the like. Aryl groups may also be fused to cycloalkyl groups to form e.g. benzo-(C_{3-7})-cycloalkyl units (e.g. indanyl, indenyl, tetralinyl, and the like). The term "Het" includes groups such as pyridinyl, thiophenyl, furanyl, pyrrolidinyl, imidazolyl, indolyl, oxadiazolyl, thiadiazolyl, triazolyl, tetrazolyl, oxatriazolyl, thiatriazolyl, pyridazinyl, morpholinyl, pyrimidinyl, pyrazinyl, quinolinyl, isoquinolinyl, piperidinyl, piperazinyl, chromanyl, thiochromanyl and the like.

Alkyl groups which R^1, R^{1a}, R^{1b}, R^{1c}, R^{1d}, R^2, R^3, R^4, R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{26a}, R^{27}, R^{28}, R^{29a}, R^{29b}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35}, R^{36}, R^y, R^f, R^g and R^h may represent, and with which Y, A^5 and R^e may be substituted; the alkyl part of alkylphenyl groups which R^{15} and R^{17} may represent; and alkoxy groups which R^2, R^3, R^4, R^c and R^d may represent, and with which A^5 and R^e may be substituted, may, when there is a sufficient number of carbon atoms, be linear or branched, saturated or unsaturated, and/or cyclic, acyclic or part cyclic/acyclic. Alkyl groups which R^c, R^d and R^e may represent, and alkylene groups which R^{27} and R^{28} (together), X_1, X_2, A^1, A^3, A^4 and A^7 may represent may, when there is a sufficient number of

carbon atoms, be linear or branched, and/or saturated or unsaturated. Cycloalkyl groups which R^c and R^d may together represent, and which R^e may include, may be branched and/or may be saturated or unsaturated.

Alkylene groups which A^5 may represent may, when there is a sufficient number of carbon atoms, be linear or branched, be saturated or unsaturated, and/or be cyclic, acyclic or part cyclic/acyclic. The C_{3-7} cycloalkyl group with which A^5 may be substituted, may be branched, saturated or unsaturated, and/or part cyclic/acyclic. This cycloalkyl group may also be attached to A^5 *via* a carbon-carbon bond or may be attached directly to the alkylene chain (i.e. to give a "spiro" compound).

Halo groups, which R^2, R^4 and R^{31} may represent, and with which R^1, R^2, R^3, R^4, R^{31}, A^5, R^e and A^7 may be substituted, include fluoro, chloro, bromo and iodo.

In the structural fragments of formulae IIa, IIb and IIc, the dots indicate the carbon atom which is bonded to the -C(O)- group and to R^1 in a compound of formula I (for the avoidance of doubt, there is no further H atom bonded to the carbon atom so indicated).

The wavy lines on the bond in the fragments of formulae IIIa, IIIb, IIIc, IVa and Ar (below) signify the bond position of the fragment.

Abbreviations are listed at the end of this specification.

Preferred compounds of the invention include those in which, when:
R^2 and R^4 do not independently represent C_{1-4} alkoxy substituted by one or more halo substituent, SR^{26}, $S(O)R^{26a}$, $S(O)_2R^{26a}$ or $N(R^{27})R^{28}$, in which

R^{27} and R^{28} independently represent $C(O)R^{30}$, or together represent C_{3-6} alkylene, thus forming a 4- to 7-membered ring, which ring is optionally substituted, on a carbon atom that is α to the nitrogen atom, with a =O group, and R^{26}, R^{26a} and R^{30} are as hereinbefore defined;

R^3 does not represent one or more optional substituents selected from C_{1-6} alkyl (optionally substituted by one or more halo group) or $N(R^{29a})R^{29b}$, in which R^{29a} and R^{29b} are as hereinbefore defined;

R^{25} does not represent $C(O)R^{30}$, in which R^{30} is as hereinbefore defined;

Y does not represent CH=CH substituted by C_{1-4} alkyl; and/or

R^{31} does not represent C_{1-4} alkyl (substituted by one or more halo group), $N(R^{32})R^{33}$, OR^{34} or SR^{35}, in which R^{32}, R^{33}, R^{34} and R^{35} are as hereinbefore defined,

(i.e. when the values of R^2, R^4, R^3 R^{25}, Y and R^{31} are other than those listed immediately above)

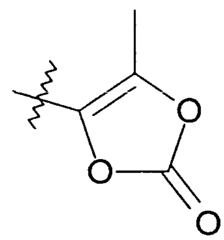
then

(i) D^1 and D^2 do not both represent H;

(ii) when D^1 or D^2 represents OR^a, then R^a does not represent H, phenyl, benzyl or C_{1-7} alkyl (which latter group is optionally interrupted by O or is optionally substituted by halo);

(iii) when D^1 or D^2 represents $C(X^{11})X^{12}R^b$ and X^{11} and X^{12} both represent O, then R^b does not represent 2-naphthyl, phenyl, C_{1-3} alkylphenyl (which latter three groups are optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy or halo); C_{1-12} alkyl (which latter group is optionally substituted by C_{1-6} alkoxy, C_{1-6} acyloxy or halo); $-[C(R^q)(R^r)]_pOC(O)R^s$, in which p is 1, 2 or 3, R^q and R^r independently represent H or C_{1-6} alkyl (provided that the total number of carbon atoms in $[C(R^q)(R^r)]_p$ does not exceed 12), and R^s represents C_{1-6} alkyl (optionally substituted by C_{1-6} alkoxy), C_{1-12} alkyl (optionally substituted by halo), C_{3-7} cycloalkyl, phenyl, naphthyl or C_{1-3} alkylphenyl (which latter four groups are optionally substituted by C_{1-6}

alkyl or halo); or $-CH_2-Ar$, in which Ar represents the structural fragment:



Compounds of the invention which may be mentioned include those in which:

R^2 and R^4 independently represent C_{1-4} alkoxy substituted by one or more halo substituents, SR^{26}, $S(O)R^{26a}$, $S(O)_2R^{26a}$ or $N(R^{27})R^{28}$, in which R^{27} and R^{28} independently represent $C(O)R^{30}$, or together represent C_{3-6} alkylene, thus forming a 4- to 7-membered ring, which ring is optionally substituted, on a carbon atom that is α to the nitrogen atom, with a =O group, and R^{26}, R^{26a} and R^{30} are as hereinbefore defined;

R^3 represents one or more optional substituents selected from C_{1-6} alkyl (optionally substituted by one or more halo group) or $N(R^{29a})R^{29b}$, in which R^{29a} and R^{29b} are as hereinbefore defined;

R^{25} represents $C(O)R^{30}$, in which R^{30} is as hereinbefore defined;

Y represents CH=CH substituted by C_{1-4} alkyl;

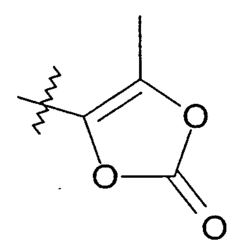
R^{31} represents C_{1-4} alkyl (substituted by one or more halo group), $N(R^{32})R^{33}$, OR^{34} or SR^{35}, wherein R^{32}, R^{33}, R^{34} and R^{35} are as hereinbefore defined.

Further compounds of the invention which may be mentioned include those in which:

(i) when one of D^1 or D^2 represents OR^a, then R^a does not represent H, phenyl, benzyl or C_{1-7} alkyl (which latter group is optionally interrupted by O or is optionally substituted by halo);

(ii) when one of D^1 or D^2 represents $C(X^{11})X^{12}R^b$ and X^{11} and X^{12} both

represent O, then R^b does not represent 2-naphthyl, phenyl, C_{1-3} alkylphenyl (which latter three groups are optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy or halo); C_{1-12} alkyl (which latter group is optionally substituted by C_{1-6} alkoxy, C_{1-6} acyloxy or halo); $-[C(R^q)(R^r)]_pOC(O)R^s$, in which p is 1, 2 or 3, R^q and R^r independently represent H or C_{1-6} alkyl (provided that the total number of carbon atoms in $[C(R^q)(R^r)]_p$ does not exceed 12), and R^s represents C_{1-6} alkyl (optionally substituted by C_{1-6} alkoxy), C_{1-12} alkyl (optionally substituted by halo), C_{3-7} cycloalkyl, phenyl, naphthyl or C_{1-3} alkylphenyl (which latter four groups are optionally substituted by C_{1-6} alkyl or halo); or $-CH_2$-Ar, in which Ar represents the structural fragment:



When n represents 2 and B represents a structural fragment of formula IIIb, preferred compounds of the invention include those wherein X^9 and X^{10} do not both represent CH_2.

Preferred compounds of formula I include those wherein:

R^1 represents OH or C_{1-4} alkyl (which latter group is optionally substituted by cyano or OH);

R_x represents a structural fragment of formula IIb or, especially, IIa;

when R_x represents a structural fragment of formula IIa, the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH-;

when R_x represents a structural fragment of formula IIa, X_1 represents optionally unsaturated C_2- or C_3-alkylene, or $-Z-A^3$ (in which Z represents O, $S(O)_m$ or $N(R^{25})$ (in which R^{25} is as hereinbefore defined or represents

C_{1-4} alkyl or $C(O)R^{30}$ and m and R^{30} are as hereinbefore defined) and A^3 represents C_1- or C_2-alkylene (which latter group is optionally unsaturated));

Y represents $(CH_2)_3$, preferably $(CH_2)_2$ and more preferably CH_2;

B represents a structural fragment of formula IIIa in which X^5, X^6, X^7 and X^8 all represent CH.

Particularly preferred compounds of the invention include those wherein, when R_x represents a structural fragment of formula IIa, X_1 represents C_3-alkylene or $-Z(CH_2)_2-$, in which Z represents $S(O)_m$, $N(R^{25})$ (in which R^{25} is as hereinbefore defined) or, especially, O.

When R_x represents a structural fragment of formula IIa, and R^2 represents at least one substituent, a preferred point of substitution is at the carbon atom which is at position E. It is preferred that at least one (and preferably two) substituents R^2 are present in a structural fragment of formula IIa.

When R_x represents a structural fragment of formula IIa, the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH- (i.e. the ring bearing R^2 is a benzo group), and R^2 represents at least one substituent, the ring is preferably substituted either at the carbon atom in the -CH=CH- group (position D) which is adjacent to the ring junction, or at the carbon atom which is at position E, or preferably at both of these sites. For example, when the fragment IIa represents a tetralin-1-yl group (i.e. the dotted lines represent bonds, A and E both represent CH, D represents -CH=CH- and X_1 represents saturated C_3-alkylene), preferred substitution positions are the 5- and 7-positions, or, preferably, di-substitution at both of these positions. Correspondingly, when the

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fragment IIa represents a chroman-4-yl, a thiochroman-4-yl, or a quinolin-4-yl, group (i.e. the dotted lines represent bonds, A and E both represent CH, D represents -CH=CH-, and X_1 represents $-Z(CH_2)_2-$, in which Z represents O, $S(O)_m$ or $N(R^{25})$), preferred substitution positions are the 8- and 6-positions, or, preferably, di-substitution at both of these positions.

Preferred optional substituents R^2 include halo, C_{1-4} alkyl, C_{1-4} alkoxy (which latter two groups are optionally substituted by one or more halo groups) or $N(R^{27})R^{28}$.

When R^1 represents OH, R_x represents an unsubstituted (by R^2 and R^4) structural fragment of formula IIc, in which X_4 represents a single bond, CH_2 or O, Y represents CH_2 or $(CH_2)_2$, R^y represents H and n represents 1, preferred compounds of the invention include those in which B does not represent a structural fragment of formula IIIb in which X^9 and X^{10} are both CH_2 and D^1 and D^2 are both H.

When D^1 and D^2 together represent a structural fragment of formula IVa, in which X^{13} is O, preferred compounds of the invention include those in which one of R^c and R^d represents H or C_{1-7} alkoxy and the other represents C_{1-7} alkyl (e.g. C_{1-4} alkyl, including linear, saturated, unsubstituted, and uninterrupted, C_{1-4} alkyl).

When D^1 or D^2 represents OR^a and R^a represents $-A^5[X^{14}]_n[C(O)]_rR^e$, and: (i) A^5 is a single bond (and thus n and r both represent 0), preferred compounds of the invention include those in which R^e is:-

(1) optionally substituted A^7-aryl, in which A^7 is preferably a single bond or C_{1-3} alkylene (e.g. C_{1-2}-alkylene) and aryl is preferably C_{6-10}-aryl, (e.g. phenyl), which A^7-aryl group is optionally substituted by one or

more halo, C_{1-6} alkoxy (e.g. C_{1-4} alkoxy, such as methoxy), C_{1-6} alkyl (e.g. C_{1-4} alkyl) or a haloalkyl (e.g. CF_3) substituent);

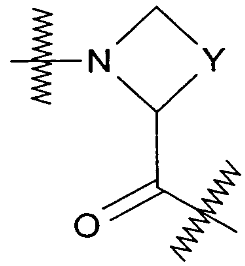
(2) H or linear, branched, optionally unsaturated, and/or cyclic, C_{1-12} alkyl (e.g. C_{3-7} alkyl), which cyclic alkyl group is optionally interrupted by an O atom and, optionally, a further O atom or $S(O)_m$ group;

(ii) A^5 is linear or branched C_{1-12} alkylene, X^{14} is O and r is 0, preferred compounds of the invention include those in which R^e is C_{1-3} alkyl or A^7-aryl, in which A^7 is a single bond and the aryl group is preferably optionally substituted phenyl.

When D^1 or D^2 represents OR^a, preferred compounds of the invention include those in which R^a is H or C_{1-4} alkyl.

When D^1 or D^2 represents $-C(=X^{11})X^{12}R^b$, in which X^{11} represents O and X^{12} represents O or S, and, in which R^b group, A^5 represents a single bond (and thus n and r both represent 0), preferred compounds of the invention include those in which R^e represents optionally unsaturated C_{1-6} (e.g. C_{1-4}) alkyl, A^7-C_{6-10}-aryl (in which A^7 represents a single bond or C_{1-2} alkylene and the C_{6-10} aryl group is preferably phenyl, which A^7-C_{6-10}-aryl group is optionally substituted by one or more halo, C_{1-4} alkyl and/or C_{1-4} alkoxy groups), or A^7-C_{3-7}-cycloalkyl (especially A^7-C_{4-5} cycloalkyl), in which A^7 represents a single bond or linear or branched C_{1-7} alkylene, and which cycloalkyl group is optionally substituted by C_{1-3} alkyl.

Compounds of formula I in which the fragment



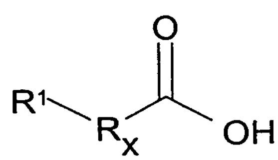
is in the S-configuration are preferred. The wavy lines on the bonds in the above fragment signify the bond position of the fragment.

Preferred compounds of formula I include the compounds of the Examples described hereinafter.

Preparation

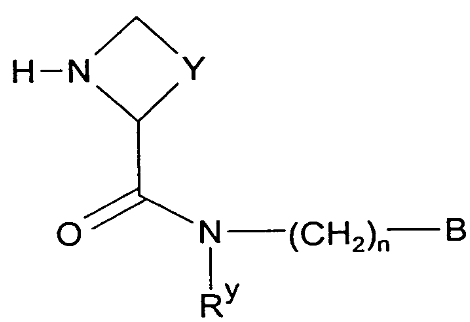
According to the invention there is also provided a process for the preparation of compounds of formula I which comprises:

(i) the coupling of a compound of formula IV,



IV

wherein R^1 and R_x are as hereinbefore defined with a compound of formula V,

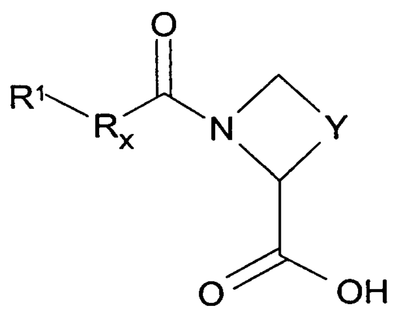


V

wherein R^y, Y, n and B are as hereinbefore defined, for example in the

presence of a coupling agent (e.g. oxalyl chloride in DMF, PyBOP, EDC, DCC, HBTU, HATU or TBTU), an appropriate base (e.g. pyridine, 2,4,6,-trimethylpyridine, 2,4,6-collidine, DMAP, TEA or DIPEA) and a suitable organic solvent (e.g. dichloromethane, acetonitrile or DMF);

(ii) the coupling of a compound of formula VI,



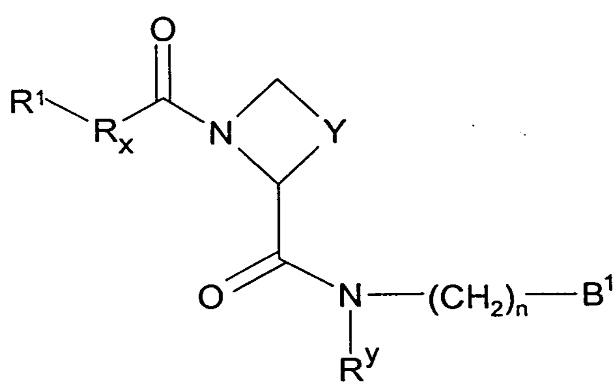
VI

wherein R^1, R_x and Y are as hereinbefore defined with a compound of formula VII,

$$H(R^y)N\text{-}(CH_2)_n\text{-}B \qquad \text{VII}$$

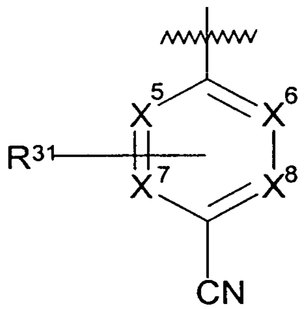
wherein R^y, n and B are as hereinbefore defined, for example in the presence of a coupling agent (e.g. oxalyl chloride in DMF, PyBOP, EDC, DCC, HBTU, HATU or TBTU), an appropriate base (e.g. pyridine, 2,4,6,-trimethylpyridine, 2,4,6-collidine, DMAP, TEA or DIPEA) and a suitable organic solvent (e.g. dichloromethane, acetonitrile or DMF);

(iii) for compounds of formula I in which D^1 or D^2 represents OR^a or NHR^a, reaction of a compound of formula VIII,

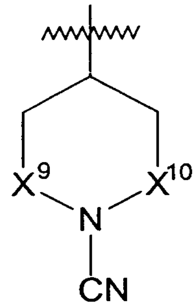


VIII

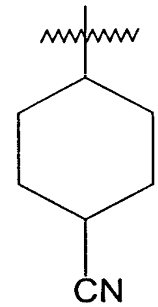
wherein B^1 represents a structural fragment of formula IIId, IIIe or IIIf



IIId



IIIe



IIIf

and R^1, R_x, Y, R^y, n, R^{31}, X^5, X^6, X^7, X^8, X^9 and X^{10} are as hereinbefore defined with a compound of formula IX,

$$H_2NX^aR^a \qquad \text{IX}$$

wherein X^a represents O or NH and R^a is as hereinbefore defined, for example at between 40 and 70°C (e.g. 60°), in the presence (optionally) of a suitable base (e.g. TEA), and an appropriate organic solvent (e.g. THF, CH_3CN, DMF or DMSO), and, optionally, wherein the compound of formula VIII is first treated with gaseous HCl, in the presence of a lower alkyl alcohol (e.g. ethanol) at, for example, 0°C;

(iv) for compounds of formula I in which D^1 or D^2 represents OR^a or NHR^a, reaction of a compound of formula I in which D^1 or D^2 (as appropriate) represents $C(O)OR^{b1}$, in which R^{b1} represents a protecting group (such as a 2-trimethylsilylethyl, a suitable alkyl (e.g. C_{1-6} alkyl), or alkylphenyl (e.g. benzyl), group) with a compound of formula IX as hereinbefore defined, for example under similar reaction conditions to those described hereinbefore for preparation of compounds of formula I (step (iii)) (the skilled person will appreciate that in such a reaction the diprotected (i.e. $C(O)OR^{b1}$ and OR^a/NHR^a protected) derivative may, in

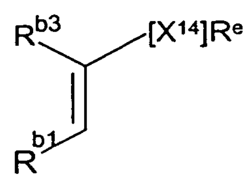
some cases, be isolated if desired, and the $C(O)OR^{b1}$ group then removed using conventional techniques);

(v) for compounds of formula I in which D^1 or D^2 represents OR^a or NHR^a, R^a represents $-A^5[X^{14}]_n[C(O)]_rR^e$, in which A^5 does not represent a single bond, and n represent 1, reaction of a compound of formula I in which D^1 or D^2 (as appropriate) represents OH or NH_2, with a compound of formula X,

$$L^1A^{5a}[X^{14}][C(O)]_rR^e \qquad \text{X}$$

wherein L^1 represents a suitable leaving group, such as lower alkoxy or halo, A^{5a} represents A^5, as hereinbefore defined except that it does not represent a single bond, and X^{14}, r and R^e are as hereinbefore defined, for example under conditions that are well known to those skilled in the art (see e.g. US 3,822,283);

(vi) for compounds of formula I in which D^1 or D^2 represents OR^a or NHR^a, R^a represents $-A^5[X^{14}]_n[C(O)]_rR^e$, in which A^5 represents C_{2-12} alkylene, which alkylene group is branched at the carbon atom that is α to the O or N atom of OR^a or NHR^a (as appropriate), and which group is optionally branched at the carbon atom that is β to that atom, n represents 1, r represents 0 and R^e is as hereinbefore defined, reaction of a compound of formula I in which D^1 or D^2 (as appropriate) represents OH or NH_2, with a compound of formula XI,

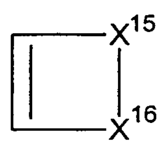


XI

or a geometrical isomer thereof, or a mixture of such geometrical isomers, in which R^{b1} and R^{b3} each represent H or an alkyl group, provided that the total number of carbon atoms provided by R^{b1} and R^{b3} does not exceed 10,

and wherein X^{14} and R^e are as hereinbefore defined, for example under conditions that are well known to those skilled in the art;

(vii) for compounds of formula I in which D^1 or D^2 represents OR^a or NHR^a, R^a represents $-A^5[X^{14}]_n[C(O)]_rR^e$, in which A^5 represents a single bond (and thus n and r both represent 0), and R^e represents A^7-C_{3-6}-cycloalkyl, in which A^7 represents a single bond, and the cycloalkyl group is interrupted by at least one O or S atom, which atom is between the carbon atom at the point of attachment to the O or NH group of OR^a or NHR^a, and a carbon atom that is α to that point of attachment, and which cycloalkyl group is optionally interrupted by one or more O or $S(O)_m$ group and/or optionally substituted by one or more =O group, reaction of a compound of formula I, in which D^1 or D^2 (as appropriate) represents OH or NH_2, with a compound of formula XII,



XII

wherein X^{15} represents O or S and X^{16} represents C_{1-4} alkylene (which alkylene group is optionally interrupted by one or more O or $S(O)_m$ group and/or optionally substituted by one or more =O group), for example under conditions that are well known to those skilled in the art;

(viii) for compounds of formula I in which D^1 or D^2 represents $C(X^{11})X^{12}R^b$, reaction of a compound of formula I in which D^1 and D^2 both represent H with a compound of formula XIII,

$$L^2\text{-}C(X^{11})X^{12}R^b \qquad \text{XIII}$$

wherein L^2 represents a suitable leaving group, such as halo or *p*-nitrophenoxy, and X^{11}, X^{12} and R^b are as hereinbefore defined, for example 0°C in the presence of a suitable base (e.g. NaOH) and an

appropriate organic solvent (e.g. THF) or water;

(ix) for compounds of formula I in which D^1 and D^2 together represent a structural fragment of formula IVa, reaction of a corresponding compound of formula I in which D^1 or D^2 represents OH or NHR^f (in which R^f is as hereinbefore defined), with a compound of formula XV,

$$(R^c)(R^d)C(R^{c1})(R^{c2}) \qquad XV$$

wherein R^{c1} and R^{c2} both represent $-OR^{c3}$, in which R^{c3} represents C_{1-3} alkyl, or together represent =O, and R^c and R^d are as hereinbefore defined, for example by using the compound of formula XV as solvent and HCl as a catalyst, at between room temperature and reflux (see e.g. *J. Org. Chem. USSR*, **21**, 177 (1985));

(x) for compounds of formula I in which one or more of X^5, X^6, X^7 and X^8 represent N-O, oxidation of a corresponding compound of formula I in which X^5, X^6, X^7 and/or X^8 (as appropriate) represent(s) N under conditions that are well known to those skilled in the art (for example in the presence of a suitable oxidising agent (e.g. *m*CPBA), at an appropriate temperature (e.g. 0°C), and in the presence of a suitable organic solvent (e.g. DCM));

(xi) for compounds of formula I in which any one of Z, X_1, R^2, R^4, A^5, A^7, R^c, R^d and/or R^e comprises or includes a S(O) or a $S(O)_2$ group, oxidation of a corresponding compound of formula I (or a compound corresponding to a compound of formula I) wherein Z, X_1, R^2, R^4, A^5, A^7, R^c, R^d and/or R^e (as appropriate) comprise(s) or include(s) a S group, in the presence of an appropriate amount of a suitable oxidising agent (e.g. *m*CPBA) and an appropriate organic solvent; or

(xii) for compounds of formula I in which D^1 and D^2 both represent H, removal of a OR^a, NHR^a or $C(=X^{11})X^{12}R^b$ group (in which R^a, R^b, X^{11} and X^{12} are as hereinbefore defined), or removal of a structural fragment of formula IVa as hereinbefore defined, from a corresponding compound of formula I (i.e. deprotection) under conditions known to those skilled in the art.

Compounds of formula IV are commercially available, are well known in the literature, or are available using known and/or standard techniques.

For example, compounds of formula IV in which R^1 represents OH may be prepared by reaction of a compound of formula XVI,

$$R_x=O \qquad \text{XVI}$$

wherein R_x is as hereinbefore defined, with:

(a) KCN, for example at 20°C in the presence of sodium bisulphite in water, followed by hydrolysis in the presence of aqueous acid (e.g. HCl), for example at 20°C in the presence of a suitable solvent (e.g. alcohol and/or water);

(b) $CHCl_3$, in the presence of aqueous base (e.g. NaOH);

(c) TMSCN, for example at 20°C in the presence of a suitable organic solvent (e.g. CH_2Cl_2), followed by hydrolysis in the presence of acid (e.g. HCl or H_2SO_4), for example at 20°C (e.g. according, or analogously, to the method described by Bigge *et al* in J. Med. Chem. (1993) **36**, 1977), followed by alkaline hydrolysis to give the free acid.

Compounds of formula IV in which R^1 represents OH may alternatively be prepared by way of a Sharpless stereoselective dihydroxylation of a compound of formula XVIA,

$$R_x=CH_2 \qquad \text{XVIA}$$

wherein R_x is as hereinbefore defined, under conditions known to those skilled in the art (e.g. at low temperature (e.g. 0°C), using, for example, the commercial reagent AD-mix-β™ in the presence of suitable solvent (e.g. *t*-butanol), followed by oxidation of the resultant intermediate (e.g. at elevated temperature (e.g. 75°C) in the presence of a stream of air and Pt/C (5%) in acetone/water).

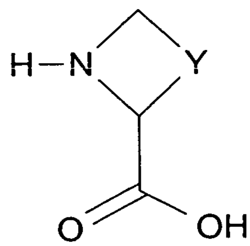
Compounds of formula IV in which R^1 represents H may be prepared from corresponding compounds of formula IV in which R^1 represents OH (or a lower alkyl ester of the acid), for example by elimination of water, followed by hydrogenation of the resultant alkene using techniques which are well known to those skilled in the art, followed by, if necessary, hydrolysis to give the free acid.

Compounds of formula IV in which R^1 represents C_{1-4} alkyl may be prepared from corresponding compounds of formula IV in which R^1 represents H (or a lower alkyl ester of the acid), for example by reaction with an appropriate alkyl halide using techniques which are well known to those skilled in the art, followed by, if necessary, hydrolysis to give the free acid.

Compounds of formula IV in which R^1 represents OR^{1d} and R^{1d} represents $C(O)R^{11}$, $SiR^{12}R^{13}R^{14}$ or C_{1-6} alkyl may be prepared by acylation, silylation or alkylation (as appropriate) of a corresponding compound of formula IV in which R^1 represents OH (or a lower alkyl ester of the acid) under conditions which are well known to those skilled in the art, followed by, if necessary, hydrolysis to give the free acid.

Compounds of formula V may be prepared by reaction of a compound of

formula XVII



XVII

wherein Y is as hereinbefore defined with a compound of formula VII as hereinbefore defined, for example under conditions such as those described hereinbefore for synthesis of compounds of formula I.

Compounds of formulae V and VII in which R^y represents C_{1-4} alkyl may be prepared by reaction of a corresponding compound of formula V or formula VII, as appropriate, in which R^y represents H with a compound of formula XVIII,

R^yHal XVIII

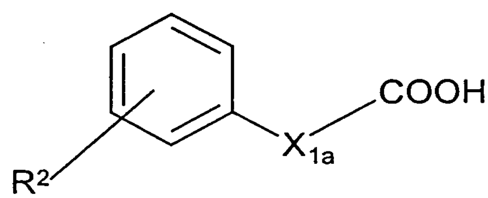
wherein Hal represents halo (e.g. Cl, Br or I) and R^y is as hereinbefore defined, for example under conditions which are well known to those skilled in the art.

Compounds of formula VI are readily available using known techniques. For example, compounds of formula VI may be prepared by reaction of a compound of formula IV as hereinbefore defined with a compound of formula XVII as hereinbefore defined, for example under conditions such as those described hereinbefore for synthesis of compounds of formula I.

Compounds of formula VIII may be prepared in accordance with peptide coupling techniques, for example in analogous fashion to the methods described hereinbefore for compounds of formula I.

Compounds of formula XVI are commercially available, are well known in the literature, or may be prepared in accordance with known techniques. For example compounds of formula XVI may be prepared as follows:

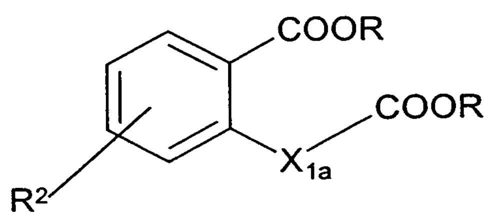
(a) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH-; X_1 represents C_{2-4} alkylene, -Z-A^3- or -C(O)-A^3-, in which Z and A^3 are as hereinbefore defined; and R^3 is absent, may be prepared by cyclisation of a compound of formula XIX,



XIX

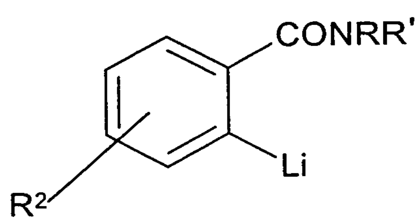
wherein X_{1a} represents C_{2-4} alkylene, -Z-A^3- or -C(O)-A^3-, and Z, A^3 and R^2 are as hereinbefore defined, using an appropriate acylating agent, for example at 100°C in the presence of polyphosphoric acid or using PCl_5 followed by $AlCl_3$, or at low temperature (e.g. 5°C) in the presence of boron trifluoride dimethyl etherate and/or trifluoroacetic anhydride and an appropriate solvent (e.g. CH_2Cl_2). Compounds of formula XIX in which X_{1a} represents C_3-alkylene or -C(O)-A^3-, in which A^3 represents C_2-alkylene, may be prepared in accordance with known techniques, for example by reaction of succinic anhydride with the corresponding phenyl lithium and, for compounds of formula XIX in which X_{1a} represents C_3-alkylene, selective reduction of the resultant ketone, under conditions which are well known to those skilled in the art. Compounds of formula XIX in which X_{1a} represents -Z-A^3- and A^3 represents C_{2-3} alkylene may be prepared as described hereinafter.

(b) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH-; X_1 represents C_{2-4} alkylene or -C(O)-A^3-, in which A^3 is as hereinbefore defined; and R^3 is absent, may alternatively be prepared by cyclisation of a compound of formula XX,



XX

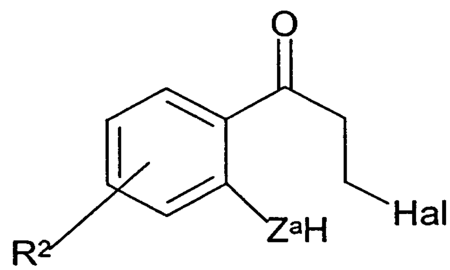
wherein R represents C_{1-6} alkyl and X_{1a} and R^2 are as hereinbefore defined, for example at 20°C in the presence of a suitable base (e.g. an alkali metal alkoxide) and an appropriate organic solvent (e.g. lower alkyl alcohol) followed by hydrolysis and decarboxylation. Compounds of formula XX may be prepared in accordance with known techniques. For example, compounds of formula XX in which X_{1a} represents C_3-alkylene or -C(O)-A^3- in which A^3 represents C_2-alkylene may be prepared by reaction of succinic anhydride with a compound of formula XXI,



XXI

wherein R′ represents C_{1-6} alkyl and R and R^2 are as hereinbefore defined and, for compounds of formula XX in which X_{1a} represents C_3-alkylene, selective reduction of the resultant ketone, followed by functional group transformations of the amide and the acid to ester groups, under conditions which are well known to those skilled in the art.

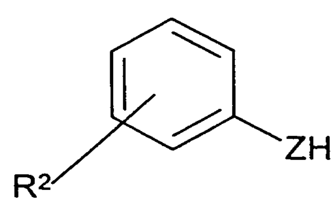
(c) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH-; X_1 represents -Z-A^3- in which A^3 represents C_2 alkylene and Z represents O or S; and R^3 is absent, may be prepared by cyclisation of a compound of formula XXII,



XXII

wherein Z^a represents O or S and Hal and R^2 are as hereinbefore defined, for example at 20°C in the presence of aqueous-ethanolic NaOH. For corresponding compounds of formula XVI in which X_1 represents -Z-A^3- and Z represents $S(O)_m$ in which m is 1 or 2, this above-mentioned cyclisation should be followed by carrying out an oxidation reaction on the cyclised product comprising an S atom, for example using *m*-chloroperbenzoic acid.

(d) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH-; X_1 represents -Z-A^3- (in which A^3 represents C_2-alkylene) or -Z-C(O)-A^1 (in which A^1 represents C_1-alkylene); and R^3 is absent, may be prepared by reaction of a compound of formula XXIII,



XXIII

wherein R^2 and Z are as hereinbefore defined, with either:-

(1) for compounds of formula XVI in which X_1 represents -Z-A^3- in which A^3 represents C_2-alkylene, a compound of formula XXIV,

$$H_2C=CH\text{-}CO_2R \qquad \text{XXIV}$$

wherein R is as hereinbefore defined, for example at 20°C in the presence of a suitable base (e.g. triethylamine or sodium ethoxide) and an appropriate organic solvent (e.g. ethanol or DMF); or

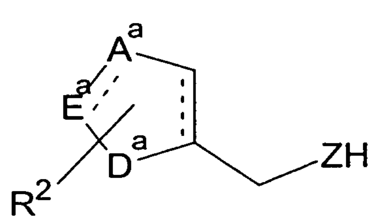
(2) a compound of formula XXV,

$$L^1\text{-}G\text{-}CH_2\text{-}CO_2R \qquad \text{XXV}$$

wherein L^1 represents a suitable leaving group (such as Cl, Br, I, mesylate or tosylate), G represents CH_2 or C(O) and R is as hereinbefore defined, for example at 20°C in the presence of a suitable base (e.g. triethylamine) and an appropriate organic solvent (e.g. THF);

followed by cyclisation under appropriate conditions (e.g. those described hereinbefore).

(e) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which the ring bearing A, E and D is a carbocyclic aromatic, or heterocyclic aromatic, ring as defined hereinbefore in respect of compounds of formula I; X_1 represents -CH_2-Z-C_{1-2} alkylene-, in which Z is as hereinbefore defined; and R^3 is absent, may be prepared by reaction of a compound of formula XXVI,



XXVI

wherein the ring bearing A^a, E^a and D^a is a carbocyclic aromatic, or heterocyclic aromatic, ring as defined hereinbefore in respect of compounds of formula I, and Z and R^2 are as hereinbefore defined, with a

compound of formula XXVII,

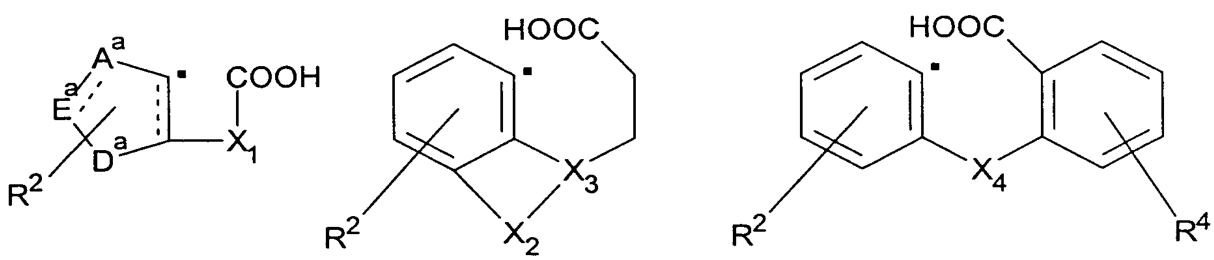
$$L^1\text{-Alk-}CO_2H \qquad \text{XXVII}$$

wherein Alk represents C_{1-2} alkylene and L^1 is as hereinbefore defined, for example at 20°C in the presence of a suitable base (e.g. sodium methoxide) and an appropriate organic solvent (e.g. THF).

(f) Compounds of formula XVI in which R_x represents a structural fragment of formulae IIb, IIc or IIa, in which latter case the ring bearing A, E and D is a carbocyclic aromatic, or heterocyclic aromatic, ring as defined hereinbefore in respect of compounds of formula I; and, in the cases when R_x represents a structural fragment of formulae IIa or IIb, R^3 is absent, may be prepared by cyclisation of a compound of formula XXIX,

$$R_{xa}\text{-}CO_2H \qquad \text{XXIX}$$

wherein R_{xa} represents a structural fragment of formula XXIXa, XXIXb or XXIXc



XXIXa XXIXb XXIXc

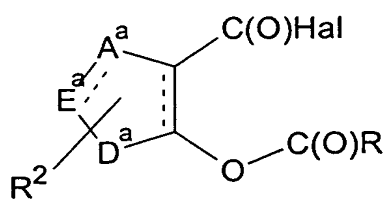
wherein, in XXIXa, the ring bearing A^a, E^a and D^a is a carbocyclic aromatic, or heterocyclic aromatic, ring as defined hereinbefore in respect of compounds of formula I, and R^2, R^4, X_1, X_2, X_3 and X_4 are as hereinbefore defined, in the presence of polyphosphoric acid, for example at 100°C. The dots adjacent to the carbon atoms in fragments of formula

XXIXa, XXIXb and XXIXc signify the point of attachment of the fragments to the CO_2H group of the compound of formula XXIX. Compounds of formula XXIX may be prepared by hydrolysis of a corresponding compound of formula XXX,

$$R_{xa}\text{-}CO_2R \qquad \text{XXX}$$

wherein R_{xa} and R are as hereinbefore defined (and in which the CO_2H in the fragments of formulae XXIXa, XXIXb and XXIXc in R_{xa} may also be replaced by CO_2R), for example under reaction conditions which are well known to those skilled in the art.

(g) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa in which the ring bearing A, E and D is a carbocyclic aromatic, or heterocyclic aromatic, ring as defined hereinbefore in respect of compounds of formula I; X_1 represents $-OCH_2-$; and R^3 is absent, may be prepared by reaction of a compound of formula XXXI,

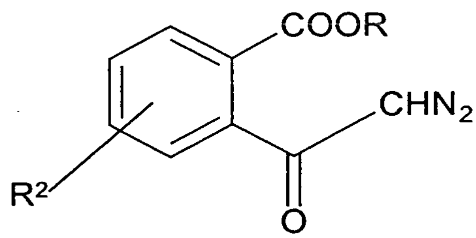


XXXI

wherein the ring bearing A^a, E^a and D^a is a carbocyclic aromatic, or heterocyclic aromatic, ring as defined hereinbefore in respect of compounds of formula I, and R^2, Hal and R are as hereinbefore defined, with diazomethane, for example at 20°C in the presence of a suitable organic solvent (e.g. diethyl ether).

(h) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH-; X_1 represents -C(O)-O-

CH_2-; and R^3 is absent, may be prepared by cyclisation of a compound of formula XXXII,



XXXII

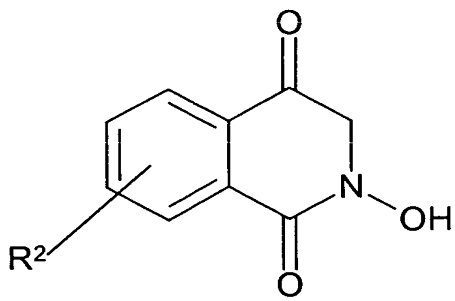
wherein R^2 and R are as hereinbefore defined, for example at -20°C in the presence of sulphuric acid and an appropriate organic solvent (e.g. methanol). Compounds of formula XXXII may be prepared by reacting a corresponding acid halide with diazomethane, for example at 20°C in the presence of a suitable organic solvent (e.g. diethyl ether).

(i) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which X_1 includes $N(R^{25})$, or IIc, in which X_4 represent $N(R^{23})$, (as appropriate), and R^{23} and R^{25} (as appropriate) represent C_{1-4} alkyl, may be prepared by reaction of a corresponding compound of formula XVI in which X_1 includes, or X_4 represents, (as appropriate) NH with a compound of formula XXXIII

R^a-Hal XXXIII

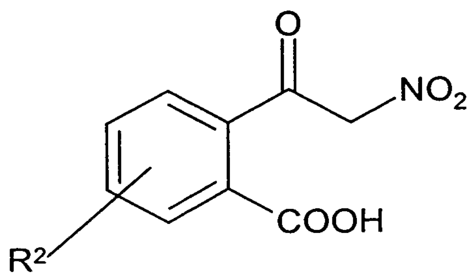
wherein R^a represents C_{1-4} alkyl and Hal is as hereinbefore defined, for example under conditions which are well known to those skilled in the art.

(j) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa, in which the dotted lines represent bonds, A and E both represent CH and D represents -CH=CH-; X_1 represents -C(O)-N(H)-CH_2-; and R^3 is absent, may be prepared by catalytic hydrogenation of an hydroxamic acid of formula XXXIV,



XXXIV

wherein R^2 is as hereinbefore defined, using an appropriate catalyst system (e.g. Pd/C) in the presence of a suitable organic solvent (e.g. methanol). Compounds of formula XXXIV may be prepared by cyclisation of a corresponding compound of formula XXXV,



XXXV

wherein R^2 is as hereinbefore defined, for example at 20°C in the presence of fuming HCl and tin dichloride.

(k) Selective oxidation of a compound of formula XXXVI,

$$H\text{-}R_x\text{-}H \qquad \text{XXXVI}$$

wherein R_x is as hereinbefore defined, for example in the presence of a suitable oxidising agent (e.g. CrO_3 or $KMnO_4$) and an appropriate solvent (e.g. water).

(l) Selective oxidation of a compound of formula XXXVII,

$$H\text{-}R_x\text{-}OH \qquad \text{XXXVII}$$

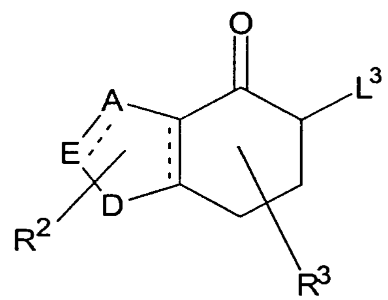
wherein R_x is as hereinbefore defined, for example in the presence of a suitable oxidising agent (e.g. MnO_2) in an appropriate organic solvent (e.g. CH_2Cl_2).

(m) Hydrolysis of an oxime formula XXXVIII,

$$R_x = N\text{-}OH \qquad \text{XXXVIII}$$

wherein R_x is as hereinbefore defined, for example by heating in the presence of acid (e.g. HCl) and an appropriate organic solvent. Compounds of formula XXXVIII may be prepared by reaction of a corresponding compound of formula XXXVI, as hereinbefore defined, with propyl nitrite, for example in the presence of HCl in ethanol.

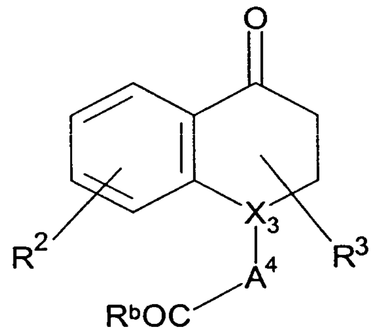
(n) Compounds of formula XVI in which R_x represents a structural fragment of formula IIa and X_1 represents $-CH_2-CH=CH-$, may be prepared by elimination of a compound of formula XXXIX,



XXXIX

wherein L^3 represents a suitable leaving group (e.g. Br or SePh) and the dotted lines, A, E, D, R^2 and R^3 are as hereinbefore defined, under appropriate reaction conditions, for example in the presence of aqueous ethanolic NaOH or hydrogen peroxide, and an appropriate organic solvent (e.g. THF).

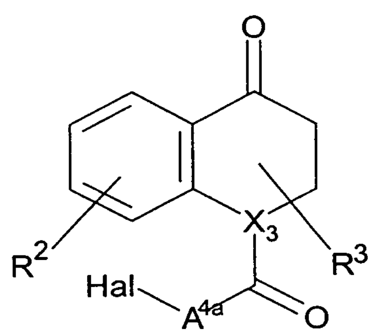
(o) Compounds of formula XVI in which R_x represents a structural fragment of formula IIb, X_2 represents $-C(O)-A^4-$ and A^4 is as hereinbefore defined, may be prepared by cyclisation of a compound of formula XL,



XL

wherein R^b represents OH, C_{1-6} alkoxy or Hal and R^2, R^3, A^4, X_3 and Hal are as hereinbefore defined, for example in the presence of polyphosphoric acid, as described hereinbefore or, in the case where R^b represents Hal, in the presence of $AlCl_3$ in nitromethane at, for example, 20°C.

(p) Compounds of formula XVI in which R_x represents a structural fragment of formula IIb and X_2 represents -A^4-C(O)- and A^4 represents C_{1-2} alkylene may be prepared by cyclisation of a compound of formula XLI,



XLI

wherein A^{4a} represents C_{1-2} alkylene and Hal, R^2, R^3 and X_3 are as hereinbefore defined.

Compounds of formulae VII, IX, X, XI, XII, XIII, XV, XVIA, XVII, XVIII, XXI, XXII, XXIII, XXIV, XXV, XXVI, XXVII, XXX, XXXI, XXXIII, XXXV, XXXVI, XXXVII, XXXIX, XL and XLI, and derivatives thereof, are either commercially available, are known in the

literature, or may be obtained either by analogy with the processes described herein, or by conventional synthetic procedures, in accordance with standard techniques, from readily available starting materials using appropriate reagents and reaction conditions (e.g. as described hereinafter).

Substituents on the aromatic and/or non-aromatic, carbocyclic and/or heterocyclic ring(s) in compounds of formulae I, IV, V, VI, VII, VIII, IX, X, XI, XIII, XVI, XVIA, XIX, XX, XII, XXII, XXIII, XXVI, XXIX, XXX, XXXI, XXXII, XXXIV, XXXV, XXXVI, XXXVII, XXXVIII, XXXIX, XL and XLI may be introduced and/or interconverted using techniques well known to those skilled in the art. For example, nitro may be reduced to amino, hydroxy may be alkylated to give alkoxy, alkoxy may be hydrolysed to hydroxy, alkenes may be hydrogenated to alkanes, halo may be hydrogenated to H, etc.

The compounds of formula I may be isolated from their reaction mixtures using conventional techniques.

It will be appreciated by those skilled in the art that in the process described above the functional groups of intermediate compounds may need to be protected by protecting groups.

Functional groups which it is desirable to protect include hydroxy, amino and carboxylic acid. Suitable protecting groups for hydroxy include trialkylsilyl or diarylalkylsilyl groups (e.g. *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl or trimethylsilyl) and tetrahydropyranyl. Suitable protecting groups for carboxylic acid include C_{1-6} alkyl or benzyl esters. Suitable protecting groups for amino, amidino and guanidino include *t*-

butyloxycarbonyl, benzyloxycarbonyl or 2-trimethylsilylethoxycarbonyl (Teoc). Amidino and guanidino nitrogens may also be protected by hydroxy or alkoxy groups, and may be either mono- or diprotected.

The protection and deprotection of functional groups may take place before or after coupling, or before or after any other reaction in the abovementioned schemes.

Protecting groups may be removed in accordance with techniques which are well known to those skilled in the art and as described hereinafter.

Persons skilled in the art will appreciate that, in order to obtain compounds of formula I in an alternative, and, on some occasions, more convenient, manner, the individual process steps mentioned hereinbefore may be performed in a different order, and/or the individual reactions may be performed at a different stage in the overall route (i.e. substituents may be added to and/or chemical transformations performed upon, different intermediates to those mentioned hereinbefore in conjunction with a particular reaction). This may negate, or render necessary, the need for protecting groups.

For example, this is particularly true in respect of the synthesis of compounds of formula I in which D^1 or D^2 (as appropriate) does not represent H. In this case, OR^a and/or $C(=X^{11})X^{12}R^b$ groups may be introduced at an earlier stage in the overall synthesis using the process steps described hereinbefore.

Accordingly, the order and type of chemistry involved will dictate the need, and type, of protecting groups as well as the sequence for

accomplishing the synthesis.

The use of protecting groups is fully described in "Protective Groups in Organic Chemistry", edited by J W F McOmie, Plenum Press (1973), and "Protective Groups in Organic Synthesis", 2nd edition, T W Greene & P G M Wutz, Wiley-Interscience (1991).

The protected derivatives of compounds of formula I may be converted chemically to compounds of formula I using standard deprotection techniques (e.g. hydrogenation). The skilled person will appreciate that certain compounds of the invention may be regarded as protected derivatives of other compounds of the invention.

Medical and pharmaceutical use

Compounds of the invention may possess pharmacological activity as such. Compounds of the invention that may possess such activity include, but are not limited to, those with a free amidine functionality as part of the structural fragment B.

However, other compounds of formula I (including those that do not possess such a free amidine functionality) may not possess such activity, but may be administered parenterally or orally, and thereafter metabolised in the body to form compounds that are pharmacologically active (including, but not limited to, corresponding free amidine compounds). Such compounds (which also include compounds that may possess some pharmacological activity, but that activity is appreciably lower than that of the active compounds to which they are metabolised to), may therefore be described as "prodrugs" of the active compounds.

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Thus, the compounds of the invention are useful because they possess pharmacological activity, and/or are metabolised in the body following oral or parenteral administration to form compounds which possess pharmacological activity. The compounds of the invention are therefore indicated as pharmaceuticals.

According to a further aspect of the invention there is thus provided the compounds of the invention for use as pharmaceuticals.

In particular, the compounds of the invention are potent inhibitors of thrombin either as such and/or (e.g. in the case of prodrugs), are metabolised following administration to form potent inhibitors of thrombin, for example as demonstrated in the tests described below.

By "prodrug of a thrombin inhibitor", we include compounds that form a thrombin inhibitor, in an experimentally-detectable amount, and within a predetermined time (e.g. about 1 hour), following oral or parenteral administration.

The compounds of the invention are thus expected to be useful in those conditions where inhibition of thrombin is required.

The compounds of the invention are thus indicated in the treatment and/or prophylaxis of thrombosis and hypercoagulability in blood and tissues of animals including man.

It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions associated with hypercoagulability and thrombo-embolic

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diseases which may be mentioned include inherited or acquired activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemi, heparin induced thrombocytopenia and defects in fibrinolysis. The compounds of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions.

The compounds of the invention are further indicated in the treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease.

Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis and pulmonary embolism, arterial thrombosis (eg in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction.

Moreover, the compounds of the invention are expected to have utility in prophylaxis of re-occlusion (ie thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

Further indications include the therapeutic and/or prophylactic treatment

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of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

In addition to its effects on the coagulation process, thrombin is known to activate a large number of cells (such as neutrophils, fibroblasts, endothelial cells and smooth muscle cells). Therefore, the compounds of the invention may also be useful for the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

Compounds of the invention that inhibit trypsin and/or thrombin may also be useful in the treatment of pancreatitis.

According to a further aspect of the present invention, there is provided a method of treatment of a condition where inhibition of thrombin is required which method comprises administration of a therapeutically effective amount of a compound of the invention, or a pharmaceutically acceptable salt thereof, to a person suffering from, or susceptible to such a condition.

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The compounds of the invention will normally be administered orally, intravenously, subcutaneously, buccally, rectally, dermally, nasally, tracheally, bronchially, by any other parenteral route or *via* inhalation, in the form of pharmaceutical preparations comprising active compound either as a free base, or a pharmaceutical acceptable non-toxic organic or inorganic acid addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated and the route of administration, the compositions may be administered at varying doses.

The compounds of the invention may also be combined and/or co-administered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2T) antagonists.

The compounds of the invention may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction.

According to a further aspect of the invention there is thus provided a pharmaceutical formulation including a compound of the invention, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Suitable daily doses of the compounds of the invention in therapeutical

treatment of humans are about 0.001-100 mg/kg body weight at peroral administration and 0.001-50 mg/kg body weight at parenteral administration.

The compounds of the invention have the advantage that they may be, or may be metabolised to compounds that may be, more efficacious, be less toxic, be longer acting, have a broader range of activity, be more potent, produce fewer side effects, be more easily absorbed than, or that they may have other useful pharmacological, physical, or chemical, properties over, compounds known in the prior art.

Biological Tests

Test A

Determination of Thrombin clotting Time (TT)

The inhibitor solution (25 μL) was incubated with plasma (25 μL) for three minutes. Human thrombin (T 6769; Sigma Chem. Co) in buffer solution, pH 7.4 (25 μL) was then added and the clotting time measured in an automatic device (KC 10; Amelung).

The clotting time in seconds was plotted against the inhibitor concentration, and the $IC_{50}TT$ was determined by interpolation.

$IC_{50}TT$ is the concentration of inhibitor in the test that doubles the thrombin clotting time for human plasma.

Test B

Determinaton of thrombin inhibition with a chromogenic, robotic assay

The thrombin inhibitor potency was measured with a chromogenic

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substrate method, in a Plato 3300 robotic microplate processor (Rosys AG, CH-8634 Hombrechtikon, Switzerland), using 96-well, half volume microtitre plates (Costar, Cambridge, MA, USA; Cat No 3690). Stock solutions of test substance in DMSO (72 µL), 1 mmol/L, were diluted serially 1:3 (24 + 48 µL) with DMSO to obtain ten different concentrations, which were analysed as samples in the assay. 2 µL of test sample was diluted with 124 µL assay buffer, 12 µL of chromogenic substrate solution (S-2366, Chromogenix, Mölndal, Sweden) in assay buffer and finally 12 µL of α-thrombin solution, (Human α-thrombin, Sigma Chemical Co.) both in assay buffer, were added, and the samples mixed. The final assay concentrations were: test substance 0.00068 - 13.3 µmol/L, S-2366 0.30 mmol/L, α-thrombin 0.020 NIHU/mL. The linear absorbance increment during 40 minutes incubation at 37°C was used for calculation of percentage inhibition for the test samples, as compared to blanks without inhibitor. The IC_{50}-robotic value, corresponding to the inhibitor concentration which caused 50% inhibition of the thrombin activity, was calculated from a log concentration vs. % inhibition curve.

Test C

Determinaton of the inhibition constant K_i for human thrombin

K_i-determinations were made using a chromogenic substrate method, performed at 37°C on a Cobas Bio centrifugal analyser (Roche, Basel, Switzerland). Residual enzyme activity after incubation of human α-thrombin with various concentrations of test compound was determined at three different substrate concentrations, and was measured as the change in optical absorbance at 405 nm.

Test compound solutions (100 µL; normally in buffer or saline containing BSA 10 g/L) were mixed with 200 µL of human α-thrombin (Sigma

Chemical Co) in assay buffer (0.05 mol/L Tris-HCl pH 7.4, ionic strength 0.15 adjusted with NaCl) containing BSA (10 g/L), and analysed as samples in the Cobas Bio. A 60 μL sample, together with 20 μL of water, was added to 320 μL of the substrate S-2238 (Chromogenix AB, Mölndal, Sweden) in assay buffer, and the absorbance change (ΔA/min) was monitored. The final concentrations of S-2238 were 16, 24 and 50 μmol/L and of thrombin 0.125 NIH U/mL.

The steady state reaction rate was used to construct Dixon plots, *i.e.* diagrams of inhibitor concentration vs. 1/(ΔA/min). For reversible, competitive inhibitors, the data points for the different substrate concentrations typically form straight lines which intercept at $x = -K_i$.

<u>Test D</u>

<u>Determination of Activated Partial Thromboplastin Time (APTT)</u>

APTT was determined in pooled normal human citrated plasma with the reagent PTT Automated 5 manufactured by Stago. The inhibitors were added to the plasma (10 μL inhibitor solution to 90 μL plasma) and incubated with the APTT reagent for 3 minutes followed by the addition of 100 μL of calcium chloride solution (0.025M) and APTT was determined in the mixture by use of the coagulation analyser KC10 (Amelung) according to the instructions of the reagent producer. The clotting time in seconds was plotted against the inhibitor concentration in plasma and the IC_{50}APTT was determined by interpolation.

IC_{50}APTT is defined as the concentration of inhibitor in human plasma that doubled the Activated Partial Thromboplastin Time.

Test E

Determination of thrombin time *ex vivo*

The inhibition of thrombin after oral or parenteral administration of the compounds of formula I, dissolved in ethanol:Solutol™:water (5:5:90), were examined in conscious rats which, one or two days prior to the experiment, were equipped with a catheter for blood sampling from the carotid artery. On the experimental day blood samples were withdrawn at fixed times after the administration of the compound into plastic tubes containing 1 part sodium citrate solution (0.13 mol per L) and 9 parts of blood. The tubes were centrifuged to obtain platelet poor plasma. The plasma was used for determination of thrombin time as described below.

The citrated rat plasma, 100 μL, was diluted with a saline solution, 0.9%, 100 μL, and plasma coagulation was started by the addition of human thrombin (T 6769, Sigma Chem Co, USA) in a buffer solution, pH 7.4, 100 μL. The clotting time was measured in an automatic device (KC 10, Amelumg, Germany).

Where a "prodrug" compound of formula I was administered, concentrations of the appropriate active thrombin inhibitor of formula I (e.g. the free amidine or guanidine compound) in the rat plasma were estimated by the use of standard curves relating the thrombin time in the pooled citrated rat plasma to known concentrations of the corresponding "active" thrombin inhibitor dissolved in saline.

Based on the estimated plasma concentrations of the active thrombin inhibitor (which assumes that thrombin time prolongation is caused by the aforementioned compound) in the rat, the area under the curve after oral and/or parenteral administration of the corresponding prodrug compound

of formula I was calculated (AUCpd) using the trapezoidal rule and extrapolation of data to infinity.

The bioavailability of the active thrombin inhibitor after oral or parenteral administration of the prodrug was calculated as below:

[(AUCpd/dose)/(AUCactive,parenteral/dose] x 100

where AUCactive,parenteral represents the AUC obtained after parenteral administration of the corresponding active thrombin inhibitor to conscious rats as described above.

<u>Test F</u>

<u>Determination of thrombin time in urine *ex vivo*</u>

The amount of the "active" thrombin inhibitor that was excreted in urine after oral or parenteral administration of "prodrug" compounds of the invention, dissolved in ethanol:Solutol™:water (5:5:90), was estimated by determination of the thrombin time in urine *ex vivo* (assuming that thrombin time prolongation is caused by the aforementioned compound).

Conscious rats were placed in metabolism cages, allowing separate collection of urine and faeces, for 24 hours following oral administration of compounds of the invention. The thrombin time was determined on the collected urine as described below.

Pooled normal citrated human plasma (100 μL) was incubated with the concentrated rat urine, or saline dilutions thereof, for one minute. Plasma coagulation was then initiated by the administration of human thrombin (T 6769, Sigma Chem Company) in buffer solution (pH 7.4; 100 μL). The

clotting time was measured in an automatic device (KC 10; Amelung).

The concentrations of the active thrombin inhibitor in the rat urine were estimated by the use of standard curves relating the thrombin time in the pooled normal citrated human plasma to known concentrations of the aforementioned active thrombin inhibitor dissolved in concentrated rat urine (or saline dilutions thereof). By multiplying the total rat urine production over the 24 hour period with the estimated mean concentration of the aforementioned active inhibitor in the urine, the amount of the active inhibitor excreted in the urine (AMOUNTpd) could be calculated.

The bioavailability of the active thrombin inhibitor after oral or parenteral administration of the prodrug was calculated as below:

[(AMOUNTpd/dose)/(AMOUNTactive,parenteral/dose] x 100

where AMOUNTactive,parenteral represents the amount excreted in the urine after parenteral administration of the corresponding active thrombin inhibitor to conscious rats as described above.

Test G

Metabolic Activation of Prodrug Compounds *in vitro*

Prodrug compounds of formula I were incubated at 37°C with liver microsomes or 10 000 g (referring to the centrifuge speed) supernatant fractions (i.e. s9 fraction) prepared from human or rat liver homogenate. The total protein concentration in the incubations were 1 or 3 mg/mL dissolved in 0.05 mol/L TRIS buffer (pH 7.4), and with the cofactors NADH (2.5 mmol/L) and NADPH (0. 8 mmol/L) present. The total volume of the incubate was 1.2 mL. The initial prodrug concentrations

were 5 or 10 μmol/L. Samples were collected from the incubate at regular intervals more than 60 minutes after the start of the incubations. Samples (25 μL) from the incubate were mixed with an equal volume of human or rat plasma and an appropriate amount of thrombin, and the clotting time (i.e. thrombin time) was measured on a coagulometer (KC 10; Amelumg). The amount of "active" thrombin inhibitor formed was estimated by the use of standard curves relating the thrombin time in pooled citrated human or rat plasma to known concentrations of the corresponding "active thrombin inhibitor".

Examples

The invention is illustrated by way of the following examples. The amino acids Pro and Aze are defined as the *S*-isomers if not otherwise specified. The examples were obtained as diastereoisomers if not otherwise specified.

Example 1

(*S*) or (*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(CO-O-CH_2-cyclopropyl)

(i) 1-Hydroxy-7-methoxytetralin-1-yl-carboxylic acid, methyl ester

The sub-title compound was prepared according to the method described by C.F.Bigge *et al* in J. Med. Chem., (1993), **36**, 1977 using 7-methoxytetralone (1.0 g; 5.67 mmol) and methanol instead of ethanol. Yield: 1.22 g (90%).

^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.05 (d, 1H), 6.80 (d, 1H), 6.65 (s, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 2.85-2.65 (m, 2H), 2.25-1.90 (m, 4H)

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(ii) 1-Hydroxy-7-methoxytetralin-1-yl-carboxylic acid

$LiOH.H_2O$ (0.41 g; 9.8 mmol) and water (4 mL) were added to a solution of 1-hydroxy-7-methoxytetralin-1-yl-carboxylic acid, methyl ester (1.16 g; 4.9 mmol; from step (i) above) in THF (10 mL). The reaction mixture was stirred at room temperature for 3 h, the THF was evaporated, and the water phase was washed with methylene chloride. The reaction mixture was acidified with HCl (2M) and some NaCl was added. After extraction with methylene chloride, the organic phase was dried and concentrated. Yield: 765 mg (70%).

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.07 (d, 1H), 6.82 (dd, 1H), 6.77 (d, 1H), 3.76 (s, 3H), 2.83-2.71 (m, 2H), 2.32-2.21 (m, 1H), 2.12-1.88 (m, 3H)

LC-MS (m/z) 221 $(M - 1)^-$

(iii) (*S*)- and (*R*)-1-Hydroxy-7-methoxytetraline-1-yl-C(O)-Aze-Pab(Z)

TBTU (0.584 g; 1.7 mmol) and DIPEA (0.200 g; 1.55 mmol) were added, in that order, to an ice-cold solution of 1-hydroxy-7-methoxytetraline-1-yl-carboxylic acid (0.345 g; 1.55 mmol, from step (ii) above) in DMF (10 mL). After stirring at 0°C for 15 minutes, H-Aze-Pab(Z) x 2HCl (0.750 g; 1.7 mmol; see international patent application WO 97/02284) and DIPEA (0.603 g; 4.65 mmol) were added and the mixture was stirred at RT for 4 days. The DMF was evaporated, and the resulting material was partitioned between water and EtOAc. The organic layer was separated, the water phase was extracted 3 times with EtOAc, and the combined organic layer was dried (Na_2SO_4) and concentrated. The product, a white powder, was further purified using HPLC (CH_3CN:0.1M ammonium acetate; 46:54), yielding 122 mg (28%) of a

faster moving fraction (Compound 1A) and 63 mg (14%) of a slower moving fraction (Compound 1B).

Compound 1A:

^{1}H-NMR (400 MHz; $CDCl_3$): (complex due to diastereomers/rotamers) δ 8.22 (t, 0.5H, rotamer); 7.94 (t, 0.5H, rotamer); 7.83 (t, 1H); 7.45-7.3 (m, 9H); 7.4 (t, 1H); 6.80 (m, 1H); 4.93 (m, 1H); 4.55 (m, 5H); 3.76 (s, 3H); 3.07-2.94 (m, 2H); 2.81 (m, 2H); 2.60 (m, 2H); 2.50 (m, 1H); 2.38 (m, 1H); 2.25 (m, 1H); 2.0-1.8 (m, 9H)

LC-MS (m/z) 571 $(M + 1)^+$

(iv) <u>(*S*)- or (*R*)-1-Hydroxy-7-methoxytetraline-1-yl-C(O)-Aze-Pab x HOAc</u>

Pd/C (5%; 50mg) was added to a solution of (*S*) or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(Z) (58 mg; 0.01 mmol; Compound 1A from step (iii) above) in EtOH (5 mL) and HOAc (5.8 μL; 0.1mmol), and the mixture was hydrogenated for 3 hours at room temperature and atmospheric pressure. The resulting mixture was filtered through Celite, the solution was concentrated, water was added and the solution was freeze dried, yielding 10 mg (98%) of the title compound. Yield 15 mg (59%).

^{1}H-NMR (400 MHz; D_2O): δ 7.65 (d, 2H); 7.47 (d, 2H); 7.16 (d, 1H); 6.90 (d, 1H); 6.71 (d, 1H); 4.91 (dd, 1H); 4.40 (m, 1H); 4.15 (m, 1H); 3.94 (m, 1H); 3.60 (s, 3H); 2.75 (m, 3H); 2.53 (m, 1H); 2.1 (m, 2H); 2.0-1.75 (m, 7H)

^{13}C-NMR (100 MHz; $CDCl_3$) δ 182.5; 178.3; 174.0

LC-MS (m/z) 437 $(M + 1)^+$

(v) *p*-Nitrophenyl-cyclopropylmethyl carbonate

Pyridine (0.43 g; 5.5 mmol) was added to an ice-cold solution of cyclopropylmethanol (0.36 g; 5.0 mmol) and *p*-nitrophenyl chloroformate (1.06 g; 5.3 mmol) in methylene chloride (10 mL), and the resultant mixture was stirred at RT overnight, whereafter the solution was washed with $KHSO_4$ (3x) and brine, dried (Na_2SO_4), and concentrated, yielding 1.2 g (97%) of the sub-title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.29 (m, 2H); 7.41 (m, 2H); 4.14 (d, 2H); 1.35-1.2 (m, 1H); 0.69 (m, 2H); 0.41 (m, 2H)

(vi) (*S*) or (*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(CO-O-CH_2-cyclopropyl)

NaOH (aq; 1.5M; 1.2 mL; 1.8 mmol) was added to a vigorously stirred solution of (*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab x HOAc (40 mg; 80 μmol; from step (iv) above) and *p*-nitrophenyl-cyclopropylmethyl carbonate (17 mg; 71 μmol; from step (v) above) in methylene chloride (5 mL) and the solution was stirred at RT for 2 hours, whereafter the organic layer was washed 3 times with NaOH (aq, 1.5M). The crude product was purified using flash chromatography (silica gel; methylene chloride → EtOAc). The fractions of interest were concentrated, dissolved in water and freeze dried, yielding 33 mg (77%) of the title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.96 (t, 1H); 7.85 (d, 2H); 7.31 (d, 2H); 7.05 (d, 1H); 6.83 (dd, 1H); 6.66 (d, 1H); 4.92 (dd, 1H); 4.6-4.4 (m, 3H); 3.99 (d, 2H); 3.83 (m, 1H); 3.75 (s, 3H); 3.04 (m, 1H); 2.80 (m, 1H); 2.5-2.7 (m, 2H); 2.25 (m, 1H); 1.8-1.2 (m, 4H); 1.24 (m, 1H); 0.59 (m, 2H); 0.33 (m, 2H)

^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons): δ 178.8; 171.4; 168.6; 165.0.

LC-MS (m/z) 536 $(M + 1)^+$

Example 2

(*S*)- or (*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(CO-O-cyclopentyl)

NaOH (aq; 1.5M; 0.44 mL; 0.66 mmol) was added to a solution of (*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab x HOAc (30 mg; 60 μmol; see Example 1(iv) above) and cyclopentyl chloroformate (9.9 mg; 66 μmol) in methylene chloride, and the mixture was stirred at RT for 3 hours, whereafter it was diluted with water, and the resultant mixture was extracted with methylene chloride (4x). The combined organic layer was dried (Na_2SO_4) and evaporated. The crude product was purified using flash chromatography (silica gel; methylene chloride → EtOAc). The fractions of interest were concentrated, yielding 16.7 mg (50%) of the title compound.

^1H-NMR (400 MHz; $CDCl_3$): δ 7.95 (t, 1H); 7.83 (d, 2H); 7.32 (d, 2H); 7.06 (d, 1H); 6.83 (dd, 1H); 6.67 (d, 1H); 5.16 (m, 1H); 4.93 (dd, 1H); 4.6-4.45 (m, 3H); 3.84 (m, 1H); 3.77 (s, 3H); 3.04 (m, 1H); 2.82 (m, 1H); 2.7-2.55 (m, 2H); 2.26 (m, 1H); 2.0-1.7 (m, 10H); 1.65-1.55 (m, 2H)

^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons): δ 178.8; 171.4; 168.5; 165.9

LC-MS (m/z) 549 $(M + 1)^+$

Example 3

(*S*)- or (*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(CO-O-cyclobutyl)

(i) *p*-Nitrophenyl-cyclobutyl carbonate

Pyridine (0.43 g; 5.5 mmol) was added to an ice-cold solution of cyclobutanol (0.36 g; 5.0 mmol) and *p*-nitrophenyl chloroformate (1.0 g; 5.0 mmol) in methylene chloride (10 mL), and the resultant mixture was stirred at RT overnight. The crude product was purified using flash chromatography (silica gel; heptane → heptane:EtOAc (90:10)). The fractions of interest were concentrated yielding 0.86 g (73 %) of the sub-title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 8.29 (m, 2H); 7.39 (m, 2H); 5.07 (m, 1H); 2.45 (m, 2H); 2.25 (m, 2H); 1.89 (m, 1H); 1.68 (m, 1H)

(ii) (*S*)- or (*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(CO-O-cyclobutyl)

NaOH (aq; 1.5M; 1 mL; 1.5 mmol) was added to a vigorously stirred solution of (*S*)- or (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab x HOAc (30 mg; 60 μmol; see Example 1(iv) above) and *p*-nitrophenyl-cyclobutyl carbonate (36 mg; 150 μmol; from step (i) above) in methylene chloride (5 mL), whereafter the solution was stirred at RT for 2.5 hours. The resultant mixture was washed 3 times with NaOH (aq; 1.5M) and 2 times with brine. The crude product was purified using flash chromatography (silica gel; methylene chloride:EtOAc (3:10)). The fractions of interest were concentrated yielding 24 mg (74%) of the title compound.

^1H-NMR (400 MHz; $CDCl_3$): δ 9.6 (br, 1H); 7.96 (t, 1H); 7.84 (d, 2H); 7.31 (d, 2H); 7.05 (d, 1H); 6.82 (dd, 1H); 6.67 (d, 1H); 5.00 (p, 1H); 4.92 (dd, 1H); 4.54 (br, 1H); 4.50 (m, 1H); 3.83 (m, 1H); 3.04 (m, 1H); 2.81 (d, 1H); 2.65-2.5 (m, 2H); 2.45-2.3 (m, 2H); 2.3-2.15 (m, 3H); 2.0-1.8 (m, 5H); 1.64 (m, 1H)

^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons) δ 178.8; 171.4; 168.7; 165.3

LC-MS (m/z) 536 $(M + 1)^+$

Example 4

(*R*,*S*)-4-Hydroxy-6-chlorochroman-4-yl-C(O)-Aze-Pab(CO-O-CH_2-cyclopropyl)

(i) 6-Chloro-4-hydroxychroman-4-yl-carboxylic acid

The sub-title compound was prepared analogously to the methods described in Example 1, steps (i) and (ii), starting from 6-chlorochromanone (2.45 g; 13.4 mmol), Me_3SiCN (1.51 g; 15.2 mmol), and ZnI_2 (40 mg; cat.). Yield: 490 mg (93%).

LC-MS (m/z) 228 $(M - 1)^-$

(ii) Boc-Aze-Pab x HCOOH

Ammonium formate (3.0 g; 50 mmol) and Pd/C (5%; 1.0 g) were added to a solution of Boc-Aze-Pab(Z) (4.7 g; 10 mmol; see international patent application WO 94/29336) in 50 mL of MeOH. Formic acid (1.0 g; 22 mmol) was added and the mixture was stirred for 30 minutes. The reaction mixture was filtered through Hyflo and the solution was concentrated. The crude product was suspended in CH_2Cl_2 (50 mL), filtered and washed with more CH_2Cl_2. The solid material was dried and used in the following step

without further purification.

(iii) Boc-Aze-Pab(Teoc)

Teoc-*p*-nitrophenyl carbonate (3.5 g; 12.3 mmol) was added to a solution of Boc-Aze-Pab x HCOOH (3.7 g; 10 mmol; from step (ii) above) in THF (100 mL) whereafter a solution of K_2CO_3 (1.8 g; 13 mmol) in water (20 mL) was added over 2 minutes. The resultant solution was stirred for 3 days, concentrated, and the remainder was taken up in EtOAc (150 mL) and NaOH (aq.; 0.5M; 50 mL). The organic layer was washed with brine (2 x 50 mL), dried (Na_2SO_4) and concentrated. The crude product was purified using flash chromatography (Si-gel; methylene chloride:acetone; 4:1). Yield 4.6 g (96%).

^{1}H-NMR (500 MHz; $CDCl_3$): δ 7.86 (d, 2H); 7.39 (d, 2H); 4.72 (bt, 1H); 4.7-4.5 (br, 2H); 3.93 (m, 1H); 3.81 (m, 1H); 2.48 (br, 2H); 1.43 (s, 9H); 0.09 (s, 9H)

(iv) H-Aze-Pab(Teoc) x HCl

A solution of Boc-Aze-Pab(Teoc) (4.6 g; 9.6 mmol; from step (iii) above) in methylene chloride (150 mL) was saturated with dry HCl. The solution was kept at RT in a stoppered flask for 10 minutes, whereafter it was concentrated. Yield 4.2 g (97%).

^{1}H-NMR (400 MHz; CD_3OD): δ 7.80 (d, 2H); 7.60 (d, 2H); 5.10 (m, 1H); 4.60 (bs, 2H); 4.15 (m, 1H); 3.97 (q, 1H); 2.86 (m, 1H); 2.57 (m, 1H); 0.11 (s, 9H)

(v) 6-Chloro-4-hydroxychroman-4-yl-C(O)-Aze-Pab(Teoc)

A solution of 6-chloro-4-hydroxychroman-4-yl-carboxylic acid (222 mg;

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1.00 mmol; from step (i) above) and HATU (370 mg, 0.97 mmol) in DMF (5 mL) was stirred at 0°C for 1.5 h, and a mixture of H-Aze-Pab(Teoc) x HCl (440 mg, 0.98 mmol; from step (iv) above) and 2,4,6-trimethylpyridine (0.48 g; 3.9 mmol) in DMF (5 mL) was added at 0°C. After stirring 3 h at 0°C the reaction mixture was concentrated, and the crude product was purified using preparative RPLC (CH_3CN:0.1M ammonium acetate; 55:45). The fractions of interest were partly concentrated and extracted with methylene chloride. The organic layer was dried (Na_2SO_4) and concentrated, yielding 350 mg (67%) of a diastereomeric mixture.

^{1}H-NMR (400 MHz; $CDCl_3$) (complex due to diasteromers/rotamers): δ 7.96 (m, 0.5H); 7.87 (bd, 1H); 7.82 (bd, 1H); 7.73 (m, 0.5H); 7.31 (m, 1H); 7.19 (dt, 1H); 7.09 (bd, 0.5H); 7.00 (bd, 0.5H); 6.88 (dd, 1H); 4.93 (m, 1H); 4.9-4.4 (m, 4H); 4.36 (m, 1H); 4.15 (bt, 1H); 3.89 (m, 0.5H); 3.74 (m, 0.5H); 3.09 (m, 1H); 2.65-2.25 (m, 4H); 1.96 (bt, 1H); 0.06 (s, 9H)

LC-MS (m/z) 588 $(M + 1)^+$

^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons) δ 176.9; 171.5; 171.3; 169.8; 155.4; 155.2

(vi) <u>(*R,S*)-6-Chloro-4-hydroxychroman-4-yl-C(O)-Aze-Pab x HOAc</u>

Bu_4NF (1.0M in THF; 0.35 mL) was added to a solution of 6-chloro-4-hydroxychroman-4-yl-C(O)-Aze-Pab(Teoc) (190 mg; 0.32 mmol; from step (v) above) in THF (20 mL) at 0°C. The solution was stirred for two days at 40°C. The solution was concentrated and the crude material was purified using preparative RPLC (CH_3CN:0.1M ammonium acetate; 25:75). Yield 115 mg (71%).

^{1}H-NMR (400 MHz; CD_3OD): δ 7.73 (m, 2H); 7.55 (m, 2H); 7.28 (dd, 1H); 7.15 (m, 1H); 6.79 (m, 1H); 4.7-4.0 (m, 6H); 2.8-2.0 (m, 4H); 1.90 (s, 3H)

LC-MS (m/z) 444 $(M + 1)^+$

^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons) δ 175.9; 175.6; 174.4; 173.1; 173.0

(vii) (*R*,*S*)-4-Hydroxy-6-chlorochroman-4-yl-C(O)-Aze-Pab(CO-O-CH_2-cyclopropyl)

NaOH (aq; 2M; 1.0 mL; 2.0 mmol) was added to a vigorously stirred solution of (*R*,*S*)-6-chloro-4-hydroxychroman-4-yl-C(O)-Aze-Pab x HOAc (31 mg; 62 μmol; from step (vi) above) and *p*-nitrophenyl-cyclopropylmethyl carbonate (39 mg, 160 μmol, see Example 1(v) above) in methylene chloride (5 mL), and the solution was stirred at RT for 2 hours. The resultant mixture was washed 3 times with NaOH (aq.; 1.5M). The crude product was purified using flash chromatography (silica gel; methylene chloride → EtOAc). The fractions of interest were concentrated yielding 25 mg (75%) of the title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): (complex due to diastereoisomers) δ 7.95 (t, 0.5H); 7.85 (d, 1H); 7.80 (m, 1.5H); 7.33 (d, 1H); 7.27 (d, 1H); 7.17 (m, 2H); 7.08 (d, 0.5H); 6.82 (m, 1H); 4.90 (m, 1H); 4.6-4.4 (m, 3H); 4.14 (m, 1H); 3.96 (d, 2H); 3.90 (m, 0.5H); 3.75 (m, 0.5H); 3.11 (m, 1H); 2.51 (m, 1H); 2.40 (m, 0.5H); 2.30 (m, 0.5H); 2.22 (m, 1H); 1.95 (m, 1H); 0.56 (m, 2H); 0.31 (m, 2H)

^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons) δ 175.2; 175.1; 171.1; 170.0; 169.9; 167.5

LC-MS (m/z) 541 $(M + 1)^+$

Example 5

(*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-CH_2-Ph(4-OMe))

(i) (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(Teoc)

The sub-title compound was prepared according to the method described in Example 4(v) above from 1-hydroxy-7-methoxytetraline-1-carboxylic acid (0.44 g; 2.0 mmol; see Example 1(ii) above), HATU (0.80 g; 2.1 mmol), H-Aze-Pab(Teoc) x HCl (1.17 g; 2.6 mmol; see Example 4(iv) above), and 2,4,6-trimethylpyridine (1.2 g; 10 mmol). The crude product (1.73 g) was purified using preparative RPLC (CH_3CN:0.1M ammonium acetate 55:45). The fractions of interest were partly concentrated and extracted with methylene chloride. The organic layer was dried (Na_2SO_4) and concentrated yielding 0.32 g (28%) of a diastereomeric mixture. Preparative RPLC (CH_3CN:0.1M ammonium acetate; 46:54) yielded two diastereomers: Compound 5A (faster moving diastereomer; 0.16 g; 28%) and Compound 5B (slower moving diastereomer; 0.16 g; 28%).

Compound 5A:

^{1}H-NMR (400 MHz; $CDCl_3$) δ 7.96 (t, 1H); 7.86 (dd, 2H); 7.36 (dd, 2H); 7.07 (d, 1H); 6.87 (dd, 1H); 6.68 (d, 1H); 4.95 (dd, 1H); 4.54 (m, 3H); 4.26 (m, 2H); 3.84 (m, 1H); 3.78 (s, 3H); 3.04 (q, 1H); 2.83 (d, 1H); 2.63 (m, 2H); 2.28 (m, 1H); 2.02-1.85 (m, 4H); 1.15 (dt, 2H); 0.08 (s, 9H)

LC-MS (m/z) 581 $(M+1)^+$

(ii) (*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(Teoc)(O-CH_2-Ph(4-OMe))

O-(4-methoxybenzyl)-hydroxylamine x HCl (78 mg; 0.41 mmol) was added to a solution of (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-

Pab(Teoc) (40 mg; 69 mmol; from step (i) above) in THF (3 mL), and the mixture was stirred at 60°C overnight. The solution was concentrated, and the crude product was purified using preparative RPLC (65% CH_3CN/0.1M ammonium acetate). The fractions of interest were concentrated, and the remaining mixture was extracted with methylene chloride. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated, yielding 35 mg (71%) of the sub-title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.79 (bt, 1H); 7.55 (s, 1H); 7.45 (d, 2H); 7.34 (m, 2H); 7.28 (s, 2H); 7.04 (d, 1H); 6.91 (m, 2H); 6.82 (dd, 1H); 6.65 (d, 1H); 5.09 (s, 2H); 4.91 (dd, 1H); 4.65 (br, 1H); 4.49 (m, 2H); 4.11 (m, 2H); 3.83 (s, 3H); 3.76 (s, 3H); 2.95 (m, 1H); 2.80 (bd, 1H); 2.60 (m, 2H); 2.25 (m, 1H); 2.0-1.8 (m, 4H); 0.94 (m, 2H); 0.00 (s, 9H)
^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons) δ 177.3; 170.6; 161.3; 156.6
LC-MS (m/z) 717 $(M+1)^+$

(iii) <u>(*R*)-1-Hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(O-CH_2-Ph(4-OMe))</u>

Bu_4NF (1M in THF; 0.1 mL; 0.1 mmol) was added to a solution of (*R*)-1-hydroxy-7-methoxytetralin-1-yl-C(O)-Aze-Pab(Teoc)(O-CH_2-Ph(4-OMe)) (34 mg; 44 mmol; from step (ii) above) in CH_3CN (2 mL) and the solution was stirred at 60°C overnight. The crude product (21.3 mg) was purified using HPLC (65% CH_3CN/0.1M ammonium acetate, yielding 10 mg (46%) of the title compound.

^{1}H-NMR (400 MHz; $CDCl_3$) δ 7.87 (br, 1H); 7.61 (d, 2H); 7.39 (d, 2H); 7.29 (d, 2H); 7.07 (d, 2H); 6.91 (d, 2H); 6.83 (dd, 1H); 6.67 (d, 1H); 5.07 (s, 2H); 4.93 (dd, 1H); 4.84 (br, 1H); 4.59 (br, 1H); 4.49 (m, 2H);

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3.82 (s, 3H); 3.77 (s, 3H); 3.02 (m, 1H); 2.82 (bd, 1H); 2.68-2.55 (m, 2H); 2.26 (m, 1H); 2.0-1.8 (m, 5H)

^{13}C-NMR (100 MHz; $CDCl_3$): (carbonyl and/or amidine carbons) δ 177.9; 170.4; 158.8

LC-MS (m/z) 573 $(M+1)^+$

Example 6

4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab

(i) O-CF_3-O'-allyl-catechol

A solution of O-CF_3-catechol (10 g, 56.2 mmol) and allyl bromide (13.6 g, 112.3 mmol) and Cs_2CO_3 (36.6 g, 112.3 mmol) in acetone (100 mL) was refluxed overnight, and the solvent was removed using a rotary evaporator. The remainder was dissolved in ether, and the resultant mixture was washed with NaOH/H_2O (2M) and water. The product (10.9 g, 89%) was pure enough to use without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 2H); 6.96 (m, 2H); 6.06 (m, 1H); 5.47 (d, 1H); 5.33 (d, 1H); 4.65 (s, 2H)

(ii) O-CF_3-O'-3-hydroxy-*n*-propyl-catechol

To a cold (ice-bath) solution of O-CF_3-O'-allyl-catechol (8.9 g, 40.8 mmol, from step (i) above) in dry THF (100 mL) under N_2 was added borane-dimethylsulphide complex (2M, 59 mL, 118.3 mmol). The temperature of the mixture was kept below 5°C and, following addition, was stirred in an ice-bath for 2 h and at ambient temperature for 1 h. The mixture was cooled and water (45 mL) was added. The mixture was stirred for a few minutes and then NaOH/H_2O (3M, 40 mL) and H_2O_2 (35%, 12.5 mL) were added. The mixture was stirred at RT for 1 h,

K_2CO_3 was added and the solution was stirred for another 5 minutes. The organic layer was separated, the THF was evaporated and ether was added. The ether solution was dried (Na_2SO_4) and evaporated, yielding 7.30 g (76%) of crude product, which was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 2H); 7.03 (d, 1H); 6.96 (m, 1H); 4.20 (m, 2H); 3.90 (m, 2H); 2.10 (m, 2H)

(iii) 3-(2-OCF$_3$-phenoxy)propionic acid

To a solution of CrO_3 (11.3 g, 112.6 mmol) and H_2SO_4 (conc., 9.5 mL) in water:acetone (21:250) was added a solution of O-CF$_3$-O'-3-hydroxy-*n*-propyl-catechol (7.0 g, 29.6 mmol, from step (ii) above) in acetone (140 mL), and the resultant mixture was stirred at RT for 2 h. *iso*-Propanol was added to destroy the remaining CrO_3, the precipitate was removed by filtration, and the acetone was evaporated. The residue was dissolved in methylene chloride and water, the organic layer was separated, and the aqueous layer was extracted twice with methylene chloride. The combined organic layer was washed with water and extracted with NaOH/H_2O (2M) whereafter the aqueous phase was washed with methylene chloride, acidified (HCl) and extracted with ether. After evaporation of the combined ethereal phase, the product, a yellow solid, was obtained (yield 4.37 g (59%)).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.21 (m, 2H); 6.99 (d, 1H); 6.94 (m, 1H); 4.28 (t, 2H); 2.87 (t, 2H)

(iv) 8-Trifluoromethoxychroman-4-one

To a cold solution of 3-(2-OCF$_3$-phenoxy)propionic acid (4.56 g, 18.2

mmol; from step (iii) above) in methylene chloride was added PCl_5 (6.45 g; 31.0 mmol) in portions, and the resultant mixture was stirred at 0°C for 1 h. To the cold solution was added $AlCl_3$ (7.29 g, 54.7 mmol) and the mixture was stirred at 0°C for 1h, and then at RT overnight. The mixture was cooled and water (50 mL) was added cautiously. After addition of further methylene chloride the organic layer was separated and the aqueous layer was extracted twice with methylene chloride. The combined organic layer was washed with water, dried (Na_2SO_4) and concentrated, yielding 4.10 g (97%) of the sub-title compound.

^1H NMR (600 MHz, $CDCl_3$) δ 7.83 (d, 1H); 7.41 (d, 1H); 6.99 (t, 1H); 4.61 (t, 2H); 2.84 (t, 2H)

(v) <u>6-Chloro-8-trifluoromethoxychroman-4-one</u>

To a solution of calcium hypochlorite (15.4 g, 72.4 mmol) in water:acetic acid (65:5) was added a solution of 8-trifluoromethoxychroman-4-one (4.2 g, 18.1 mmol; from step (iv) above) in acetonitrile (20 mL), whereafter the reaction mixture was stirred overnight. The mixture was diluted with water and extracted with ether (3 times) and EtOAc (once). The combined organic layer was washed with water, dried (Na_2SO_4) and evaporated, yielding 4.2 g (87%) of the sub-title compound.

^1H NMR (300 MHz, $CDCl_3$) δ 7.83 (m, 1H); 7.43 (m, 1H); 4.64 (t, 2H); 2.90 (t, 2H)

(vi) <u>4-Cyano-4-OTMS-6-chloro-8-trifluromethoxychromane</u>

A solution of 6-chloro-8-trifluoromethoxychroman-4-one (2.0 g, 7.5 mmol, from step (v) above), TMSCN (0.8 g, 8.3 mmol) and ZnI_2 (cat.) in methylene chloride (50 mL) was stirred at RT for 2 days, whereafter the

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crude product was used directly for the next step.

^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, 1H); 7.28 (sh, 1H); 4.52-4.38 (m, 2H); 2.52-2.38 (m, 2H); 0.26 (s, 9H)

(vii) 4-Hydroxy-6-chloro-8-trifluoromethoxy-4-carbimidic acid, methyl ester

The product solution from the step (vi) above was added drop-wise to an ice cold saturated solution of HCl in MeOH, whereafter the resultant mixture was stirred overnight. The solvent was removed *in vacuo*, and the resultant material was used directly to the next step.

(viii) 4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl carboxylic acid, methyl ester

The crude product from step (vii) above was dissolved in THF (100 mL), and H_2SO_4 (0.5M, 100 mL) was added, whereafter the mixture was left standing at RT for 3 days. The solution was partially concentrated and the aqueous solution was extracted with ether (3x). The combined organic phase was dried (Na_2SO_4) and concentrated. The crude product was used directly in the next step.

(ix) 4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl carboxylic acid

A solution of 4-hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl carboxylic acid, methyl ester in *iso*-propanol (50 mL) was added to KOH/H_2O (20%, 60 mL) and the mixture was refluxed overnight. The resultant solution was partially concentrated, and the remainder was acidified with H_2SO_4 (10%). The very turbid mixture was extracted with ether (3x), and the combined organic phase was dried (Na_2SO_4) and

concentrated. The crude product was purified *via* prep-HPLC (CH_3CN:0.1M ammonium acetate (30:60)). The fractions of interest were partly concentrated and extracted with ether. The combined organic layers were washed with water, dried (Na_2SO_4), and concentrated, yielding 0.24 g (10% over steps (vi) – (ix)).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.18 (s, 1H); 7.11 (s, 1H); 4.51 (m, 1H); 4.27 (m, 1H); 2.47 (m, 1H); 2.15 (m, 1H)

(x) 4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab(Teoc)

To a solution of 4-hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl carboxylic acid (0.24 g, 0.77 mmol; from step (ix) above), was added H-Aze-Pab(Teoc) (0.38 g, 0.84 mmol, see Example 4(iv) above), PyBOP (0.44 g, 0.84 mmol) in DMF (7 mL), and DIPEA (0.40 g, 3.07 mmol). The resultant mixture was stirred overnight, poured into water and then extracted with EtOAc (3x). The combined organic layer was washed with water, dried (Na_2SO_4) and concentrated. The crude product was purified by flash chromatography (methylene chloride:THF (7:3)) to yield 0.22 g (43%) of the sub-title product as a diastereomeric mixture.

^{1}H NMR (600 MHz, $CDCl_3$) (complex due to diastereomers/rotamers) δ 7.79 (d, 1H); 7.76 (d, 1H); 7.67 (t, 1H); 7.31 (d, 1H); 7.25 (d, 1H); 7.18 (s, 1H); 7.04 (d, 0.5H); 7.00 (d, 0.5H); 4.87 (m, 1H); 4.60-4.36 (m, 3H); 4.22-4.13 (m, 3H); 3.91 (m, 0.5H); 3.76 (m, 0.5H); 3.14 (m, 1H); 2.55-2.20 (m, 3H); 1.98 (m, 1H); 1.07 (m, 2H); 0.03 (s, 9H)
LC-MS (m/z) 671 $(M+1)^+$

(xi) 4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab

A solution of 4-hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab(Teoc) (106 mg, 0.16 mmol; from step (x) above) in TFA (2 mL) was left at RT for 30 min, whereafter the solution was concentrated *in vacuo*. The product was dissolved in a minimal amount of water and freeze dried overnight, yielding 100 mg (99%) of the title compound with a purity of 96%.

^1H NMR (400 MHz, CD_3OD) (complex due to diastereomers/rotamers) δ 7.74 (m, 2H); 7.60-7.50 (m, 2H); 7.38 (d, 0.5H); 7.30 (d, 0.5H); 7.24 (m, 1H); 4.87 (sh, 1H); 4.65-4.40 (m, 4H); 4.35-4.00 (m, 2H); 2.75 (m, 0.5H); 2.60 (m, 1H); 2.42 (m, 1H); 2.37-2.05 (m, 2.5H)

LC-MS (m/z) 527 $(M+1)^+$

^{13}C NMR (carbonyl and/or amidine carbons; 100 MHz, CD_3OD) δ 174.1; 173.7; 172.0; 171.9; 166.9

Example 7

4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab(OMe)

(i) 4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab(OMe)(Teoc)

A solution of 4-hydroxy-6-chloro-8-trifluoromethoxychroman4-yl-C(O)-Aze-Pab(Teoc) (40 mg, 0.06 mmol; see Example 6(x) above) and O-methylhydroxylamine (30 mg, 0.36 mmol) in THF (5 mL) was heated at 65°C for 2 days, whereafter the solvent was removed *in vacuo* and the crude product was purified by prep-HPLC (CH_3CN:0.1M ammonium acetate 50:50-70:30). The fractions of interest were partly concentrated and extracted with EtOAc. The combined organic phase was dried (Na_2SO_4) and concentrated to dryness, giving the sub-title compound (22

mg, 53%).

^{1}H NMR (600 MHz, $CDCl_3$) (complex due to diastereomers/rotamers) δ 7.64 (bt, 0.5H); 7.57 (d, 1H); 7.52 (d, 1H); 7.48 (d, 1H); 7.43 (bt, 0.5H); 7.34 (d, 1H); 7.29 (d, 1H); 7.23 (s, 1H); 7.09 (d, 0.5H); 7.06 (d, 0.5H); 4.90 (m, 1H); 4.75 (b, 1H); 4.61-4.44 (m, 3H); 4.22-4.12 (m, 3H); 3.96 (s, 3H); 3.90 (m, 0.5H); 3.76 (m, 0.5H); 3.15 (q, 0.5H); 3.05 (m, 0.5H); 2.59 (m, 1H); 2.44 (m, 0.5H); 2.36 (m, 0.5H); 2.25 (m, 1H); 2.02 (dd, 1H); 1.67 (b, 1H); 0.97 (m, 1H); 0.02 (d, 9H)

(ii) 4-Hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab(OMe)

A solution of 4-hydroxy-6-chloro-8-trifluoromethoxychroman-4-yl-C(O)-Aze-Pab(OMe)(Teoc) (22 mg, 0.03 mmol, from step (i) above) in TFA (3.0 mL) was stirred for 15 minutes, whereafter the solution was concentrated. The crude product was dissolved in water, and the product was freeze dried, yielding 20 mg (95%) of the title compound.

^{1}H NMR (600 MHz, CD_3OD) (complex due to diastereomers/rotamers) δ 7.63 (m, 2H); 7.53 (m, 2H); 7.36 (m, 0.5H); 7.28 (m, 0.5H); 7.22 (m, 1H); 4.82 (dd, 1H); 4.62-4.00 (m, 6H); 3.92 (s, 3H); 2.71 (m, 0.5H); 2.55 (m, 0.5H); 2.40 (m, 1H); 2.27 (m, 0.5H); 2.20 (m, 0.5H); 2.10 (m, 1H)

LC-MS (m/z) 557 $(M+1)^+$

^{13}C NMR (carbonyl and/or amidine carbons; 100 MHz, CD_3OD) δ 173.9; 173.6; 171.8; 160.6

Example 8

(*S*)- or (*R*)-4-Hydroxy-6-chloro-8-difluoromethoxychroman-4-yl-C(O)-Aze-Pab x HOAc

(i) O-CHF_2-O´-allyl-catechol

To a solution of *iso*-propanol (120 mL) and KOH/H_2O (30%, 120 mL) was added O-allyl-catechol (26 g, 173 mmol). The resultant mixture was warmed to 70°C, and a stream of chlorodifluoromethane was bubbled through the solution for 45 minutes. The mixture was stirred for 30 minutes at 70°C and then at room temperature overnight. The mixture was diluted with water (1000 mL), and extracted with ether (3x). The combined organic phases were washed with NaOH/H_2O (2M) and water, then dried (Na_2SO_4) and concentrated, yielding 20.5 g (59%) of the sub-title compound, which was used without further purification.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.15(m, 2H); 6.93 (m, 2H); 6.30-6.80 (t, 1H); 5.95-6.10 (m, 1H); 5.25-5.45 (dd, 2H); 4.55-4.60 (d, 2H)

(ii) O-CHF_2-O´-3-hydroxy-*n*-propyl-catechol

To a cold (ice-bath) solution of O-CHF_2-O´-allyl-catechol (20.5 g, 102.4 mmol, from step (i) above) in dry THF (200 mL) under N_2 was added borane-dimethylsulphide complex (2M, 149 mL, 298 mmol). The temperature of the mixture was kept at 5°C and, following addition, was stirred at this temperature for 2 h, and then at room temperature for 1 h. The mixture was cooled and water (111 mL) was added. The mixture was stirred for a few minutes and then NaOH/H_2O (3M, 102 mL) and H_2O_2 (35%, 31 mL) were added. The mixture was stirred (ice-bath) for a few minutes and then at room temperature for 1 h. K_2CO_3 (77 g, 557 mmol) was added and the mixture was stirred for a few minutes. The organic

layer was separated, the THF was evaporated and ether was added. The ether solution was washed with water (3x), then dried (Na_2SO_4) and concentrated, yielding 16.2g (72%) of the sub-title compound, which was used without further purification.

^1H-NMR (400 MHz; $CDCl_3$): δ 7.10-7.17 (m, 2H); 6.86-6.98 (m, 2H); 6.32-6.71 (t, 1H); 4.11 (t, 2H); 3.81 (t, 2H); 2.86 (s, 1H); 2.1 (m, 2H)

(iii) 3-(2-$OCHF_2$-phenoxy)propionic acid

To a cold (ice-bath) solution of CrO_3 (27.8 g, 278 mmol) in water (53 mL) was added H_2SO_4 (conc., 23.5 mL). The mixture was cautiously (ice-bath) added to acetone (500 mL). To the resultant mixture was added, dropwise over 2 h, a solution of O-CHF_2-O´-3-hydroxy-*n*-propyl-catechol (16.0 g, 73 mmol, from step (ii) above) in acetone (350 mL), whereafter the reaction mixture was stirred at room temperature overnight. The precipitate was removed by filtration and the filter cake washed with acetone. The filtrate was concentrated *in vacuo*, the residue was dissolved in methylene chloride, and washed with water (3x). The filter-cake was dissolved in the combined water solutions, and the resultant solution was extracted with methylene chloride (2x). The combined methylene chloride phases were dried (Na_2SO_4) and concentrated *in vacuo* to afford 15.2 g (89%) of the sub-title compound.

^1H-NMR (400 MHz; $CDCl_3$): δ 9.64 (s, 1H); 7.12-7.20 (m, 2 H); 6.91-7.02 (m, 2H) 6.30-6.70 (t, 1H); 4.29 (t, 2H); 2.88 (t, 2H)

(iv) 8-Difluoromethoxychroman-4-one

To a cold (ice-bath) solution of 3-(2-$OCHF_2$-phenoxy)propionic acid (12.5 g, 53.8 mmol, from step (iii) above) in methylene chloride (175 mL)

under N_2 was added, dropwise, boron trifluoride dimethyl etherate (12.5 mL, 136 mmol) and then trifluoroacetic anhydride (20.0 mL, 143.8 mmol). The reaction mixture was stirred at 5°C for 1 h. The mixture was cooled and water (175 mL) was added cautiously. The organic layer was separated, washed with $NaHCO_3$/aq, dried (Na_2SO_4) and evaporated. The crude product was chromatographed on silica gel, eluting with methylene chloride, to afford 6.3 g (55%) of the sub-title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.70-7.74 (dd, 1H); 7.32 - 7.37 (dd, 1H); 6.93-7.00 (t, 1H); 6.41- 6.81 (t, 1H); 4.58-4.64 (t, 2H); 2.80-2.85 (t, 2H)

(v) <u>6-Chloro-8-difluoromethoxychroman-4-one</u>

To a solution of calcium hypochlorite (29.4 g, 137.8 mmol) in water:acetic acid (125:9.5) was added a solution of 8-difluoromethoxychroman-4-one (7.4 g, 34.6 mmol, see step (iv) above) in acetonitrile (20 mL), whereafter the reaction mixture was stirred overnight. The mixture was diluted with water and extracted with ether (3x) and with EtOAc (1x). The combined organic layers were washed with water (3x), dried (Na_2SO_4) and evaporated, yielding 8.0 g (93%) of the sub-title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.70-7.73 (d, 1H); 7.33-7.37 (d, 1H); 6.40-6.80 (t, 1H); 4.63 (t, 2H); 2.86 (t, 2H)

(vi) <u>4-Methylene-6-chloro-8-difluoromethoxychromane</u>

To a solution of dimethyl methylphosphonate (8.9 g, 71.1 mmol) in dry THF (55 mL) under N_2 was added, dropwise over 1 h at -70°C, *n*-butyllithium in hexane (1.6M, 49.2 mL). The mixture was stirred for 30 minutes at -70°C, whereafter a solution of 6-chloro-8-

difluoromethoxychroman-4-one (6.8 g, 27.35 mmol, from step (v) above) in dry THF (15 mL) was added, dropwise over 30 minutes at -70°C. The reaction mixture was stirred for 2 h at -70°C, whereafter NH_4Cl (aq., sat., 110 mL) was added, and then water (50 mL). The layers were separated and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated. The residue was dissolved in DMF (85 mL). To the solution was added anhydrous potassium carbonate (27.4 g, 201.0 mmol) and water (3.6 ml). The mixture was warmed for 2 h at 120°C (oil-bath). After the mixture had reached room temperature water (80 mL) was added. The mixture was extracted with EtOAc. The combined organic layers were washed with citric acid (aq., 10%) and with brine, dried (Na_2SO_4) and evaporated. The crude product was chromatographed on silica gel, eluting with heptane:methylene chloride (8:2), to afford 3.2 g (47%) of the sub-title compound.

^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.38 (d, 1H); 7.03 (d, 1H); 6.33-6.74 (t, 1H); 6.52 (s, 1H); 4.99 (s, 1H); 4.27 (t, 2H); 2.66 (t, 2H)

(vii) <u>(*S*)- or (*R*)-4-Hydroxy-4-hydroxymethyl-6-chloro-8-difluoromethoxychromane</u>

To a mixture of *tert*-butanol (46 mL) and water (46 mL) was added AD-mix-β (18.6 g). The mixture was cooled to 0°C. 4-Methylene-6-chloro-8-difluoromethoxychromane (3.2g 12.97 mmol, see step (vi) above) in *tert*-butanol (11mL) and water (11mL) was added. The mixture was stirred at 0°C for 24 h. Sodium sulfite (19.0 g, 150.74 mmol) was added, and the mixture was allowed to warm to room temperature and stirred for 1 h. The layers were separated and the aqueous phase was extracted with EtOAc (2x). The combined ethyl acetate phases were dried (Na_2SO_4) and

evaporated. The crude product was chromatographed on silica gel, eluting with methylene chloride:ethyl acetate (4:6), to afford 3.2 g (88%) of the sub-title compound.

^{1}H-NMR (400 MHz; CD_3OD): δ 7.38 (d, 1H); 7.07 (d, 1H); 6.51-6.74 (t, 1H); 4.86 (s, 2H); 4.3 (m, 2H); 3.70 (dd, 2H); 2.25-2.35 (m, 1H); 1.91–2.02 (m, 1H)

(viii) <u>(*S*)- or (*R*)-4-Hydroxy-6-chloro-8-difluoromethoxychroman-4-yl carboxylic acid</u>

To a solution of (*S*)- or (*R*)-4-hydroxy-4-hydroxymethyl-6-chloro-8-difluoromethoxychromane (3.2 g, 11.4 mmol, from step (vii) above) in acetone (25 mL) was added non-ionized water (110 mL), then sodium hydrogencarbonate (2.13 g, 22.2 mmol) and Pt/C 5%, 58% water (2.13 g). A stream of air was bubbled through the solution with stirring at 75°C (oil-bath) overnight. The solution was filtered through Celite and the filter-cake was washed with water. The acetone-water solution was acidified (HCl, 2M) to pH 2, saturated with NaCl and extracted with EtOAc (3x). The combined ethyl acetate phases were washed with water (2x) and with brine, then dried (Na_2SO_4) and concentrated. The residue was dissolved in ether. The ether solution was washed with water (3x) and concentrated, yielding 2.4 g (71%) of the sub-title compound.

^{1}H-NMR (400 MHz; CD_3OD): δ 7.19 (d, 1H); 7.11 (d, 1H); 6.54–6.75 (t, 1H); 4.90-5.30 (s, 2H); 4.43-4.52 (m, 1H); 4.22-4.32 (dt, 1H); 2.44-2.55 (dt, 1H); 2.06-2.16 (dd, 1H)

$[\alpha]_D^{20}$ = -20°C (c = 1%, MeOH)

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(ix) <u>(*S*)- or (*R*)-4-Hydroxy-6-chloro-8-difluoromethoxychroman-4-yl-C(O)-Aze-Pab(Teoc)</u>

To ethyl acetate (20 mL) saturated with HCl was added Boc-Aze-Pab(Teoc) (0.33 g, 0.66 mmol, see Example 4(iii) above). The mixture was kept at room temperature for 15 minutes, whereafter it was concentrated. To the residue dissolved in DMF (4.5 mL) was added (*S*)- or (*R*)-4-hydroxy-6-chloro-8-difluoromethoxychroman-4-yl carboxylic acid (0.195 g, 0.66 mmol, from step (viii) above), then PyBOP (0.36 g, 0.68 mmol) and DIPEA (0.33 g, 0.68 mmol). The mixture was stirred at room temperature for 2 h, diluted with water (175 mL) and sodium hydrogencarbonate was added to give a pH of 9. The mixture was then extracted with EtOAc (3x). The combined ethyl acetate phases were washed with water and with brine, and then dried (Na_2SO_4) and concentrated. The crude product was further purified using preparative HPLC (CH_3CN:0.1 M ammonium acetate, 60:40). The fractions of interest were concentrated. The residue was dissolved in water. The aqueous phase was extracted with EtOAc (3x). The combined ethyl acetate phases were washed with water and with brine, and then dried (Na_2SO_4) and concentrated, yielding 0.3 g (68%) of the sub-title compound.

^{1}H-NMR (400 MHz; CD_3OD): δ 7.80 (m, 2H); 7.09-7.44 (m, 3H); 6.52-7.00 (dt, 1H); 5.48 (m, 1H); 4.86 (s, 4H); 3.80-4.60 (m, 8H)); 1.80-2.80 (m, 4H); 1.22 (t, 1H); 1.08 (t, 2H); 0.07 (s, 9H)

(x) <u>(*S*)- or (*R*)-4-Hydroxy-6-chloro-8-difluoromethoxychroman-4-yl-C(O)-Aze-Pab x HOAc</u>

To a cold solution of (*S*)- or (*R*)-4-hydroxy-6-chloro-8-difluoromethoxychroman-4-yl-C(O)-Aze-Pab(Teoc) (0.30 g, 0.459 mmol, from step (ix) above) in methylene chloride (1 mL) was added TFA (10 mL). The mixture was stirred for 1 h, and then concentrated carefully *in vacuo*. The crude product was further purified using preparative HPLC (CH_3CN:0.1 M ammonium acetate, 30:70). The fractions of interest were concentrated. The product was dissolved in a minimal amount of CH_3CN/water and freeze dried (2x), yielding 0.24 g (92%) of the title compound.

^{1}H-NMR (400 MHz; CD_3OD): δ 7.68-7.78 (dd, 2H); 7.49-7.57 (dd, 2H); 7.27 (d, 1H); 7.09-7.15 (dd, 1H); 6.56 –6.94 (t, 1H); 5.51-5.54 (m, 1H); 4.90-5.02 (m, 8H); 3.98-4.62 (m, 6H); 2.08-2.80 (m, 5H); 1.91 (s, 3H).
^{13}C NMR (400 MHz; CD_3OD): carbonyl and/or amidine carbons 179.30; 175.93; 175.37; 174.31; 173.04; 168.11.
MS (m/z) 509 $(M+1)^+$

Example 9

<u>(*S*)- or (*R*)-4-Hydroxy-6-chloro-8-difluoromethoxychroman-4-yl-C(O)-Aze-Pab(OMe)</u>

To a solution of (*S*)- or (*R*)-4-hydroxy-6-chloro-8-difluoromethoxychroman-4-yl carboxylic acid (0.065 g, 0.22 mmol, see Example 8(viii) above) in DMF (1.5 mL) was added H-Aze-Pab(OMe) x 2HCl (0.060 g, 0.23 mmol, see international patent application WO 98/57932), then PyBOP (0.12 g, 0.23 mmol) and DIPEA (0.11 g, 0.85 mmol). The mixture was stirred at room temperature for 1.5 h and then evaporated. The residue was dissolved in water (50 mL) and sodium

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hydrogencarbonate was added to give a pH of 9. The mixture was extracted with EtOAc (3x). The combined ethyl acetate phases were washed with $NaHCO_3$/aq (2x) and with water, dried (Na_2SO_4) and concentrated. The crude product was further purified using preparative HPLC (CH_3CN:0.1 M ammonium acetate, 50:50). The fractions of interest were concentrated. The product was dissolved in a minimal amount of CH_3CN/water and freeze dried (2x), yielding 0.080 g (67%) of the title compound.

^{1}H-NMR (400 MHz; CD_3OD): δ 7.44 (d, 1H); 7.10-7.26 (m, 2H); 6.91-7.01 (d, 1H); 6.40–6.80 (t, 1H); 5.31 (m, 1H); 4.67 (s, 2H); 4.20-4.40 (m, 2H); 3.80-4.15 (m, 2H); 3.66 (s, 1H); 2.97-3.01 (m, 4H); 2.50-2.64 (m, 1H); 1.87-2.41 (m, 4H); 1.67 – 1.71 (t, 3H)
^{13}C NMR (400 MHz; CD_3OD): carbonyl and/or amidine carbons 175.87; 175.31; 174.04; 172.73
MS (m/z) 539 $(M+1)^+$

Example 10

The title compounds of Examples 6 and 8 were tested in Test A above and were found to exhibit an $IC_{50}TT$ value of less than 0.1 μM.

Example 11

The title compounds of Examples 1 to 5, 7 and 9 were tested in Test E above and were all found to exhibit oral and/or parenteral bioavailability in the rat as the corresponding active inhibitor (free amidine).

Example 12

The title compounds of Examples 1 to 5 were tested in Test G above and all exhibited formation of the corresponding active inhibitor (free

amidine).

Abbreviations

Ac =	acetyl
AcOH =	acetic acid
Aze =	azetidine-2-carboxylate
AzeOH =	azetidine-2-carboxylic acid
Bzl =	benzyl
DIPEA =	diisopropylethylamine
DMAP =	4-(N,N-dimethyl amino) pyridine
DMF =	dimethylformamide
DMSO =	dimethylsulphoxide
EDC =	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
Et =	ethyl
ether =	diethyl ether
EtOAc =	ethyl acetate
EtOH =	ethanol
h =	hours
HATU =	O-(azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HBTU =	[N,N,N',N'-tetramethyl-O-(benzotriazol-1-yl)uronium hexafluorophosphate]
HCl(g) =	hydrogen chloride gas
HOAc =	acetic acid
LC =	liquid chromatography
Me =	methyl
MeOH =	methanol

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Pab = *para*-amidinobenzylamino

H-Pab = *para*-amidinobenzylamine

PyBOP = (benzotriazole-1-yl-oxy)tripyrrolidinophosphonium hexafluorophosphate

RPLC = reverse phase high performance liquid chromatography

RT = room temperature

TBTU = [N,N,N',N'-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate]

TEA = triethylamine

Teoc = 2-(trimethylsilyl)ethoxycarbonyl

THF = tetrahydrofuran

TLC = thin layer chromatography

Val = L-valine

Z = benzyloxycarbonyl

Prefixes *n*, *s*, *i* and *t* have their usual meanings: normal, secondary, iso and tertiary.